AR201-13450B

Robust Summaries for Benzyl Derivatives

Benzaldehyde	**CAS No. 100-52-7**
***p*-Methoxybenzaldehyde**	**CAS No. 123-11-5**
***m*-Methoxy-*p*-hydroxybenzaldehyde**	**CAS No. 121-33-5**
Benzyl acetate	**CAS No. 140-11-4**
Benzyl benzoate	**CAS No. 120-51-4**
Methyl benzoate	**CAS No. 93-58-3**
Methyl *p*-methylbenzoate	**CAS No. 99-75-2**
Methyl 2-hydroxybenzoate	**CAS No. 119-36-8**
Pentyl 2-hydroxybenzoate	**CAS No. 2050-08-0**
Benzyl 2-hydroxybenzoate	**CAS No. 118-58-1**

FFHPVC Aromatic Consortium Registration Number [redacted]

Submitted to the EPA under the HPV Challenge Program by:
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List of Member Companies

BASF

Eastman Chemical Company

Firmenich, Incorporated

Givaudan Corporation

Haarmann & Reimer Corporation

International Flavor & Fragrances, Inc.

KoSa

Lyondell Chemical Company

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Rhodia Incorporated

Sumitomo Chemical Company

Robust Summaries for the Benzyl Derivatives

Preface:

IUCLID Data Set

Existing Chemical	ID: 100-52-7
CAS No.	100-52-7
EINECS Name	benzaldehyde
EC No.	202-860-4
TSCA Name	Benzaldehyde
Molecular Formula	C7H6O

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo:	Benzyl Derivatives
Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = -56.5 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (44)

Value: = -26 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (3)

2.2 Boiling Point

Value: = 179 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (3)

Value: = 179 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (11)

2.4 Vapour Pressure

Value: = 1.19 hPa at 20 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (12)

Value: = 1.34 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated/Mean of Antoine & Grain method
Test condition: Calculated based on a measured boiling point of 179 C.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (50)

Value: = 1.69 hPa at 25 degree C

Method: other (measured)
Year: 1975
GLP: no data
Test substance: other TS

Method: Measured
Test substance: Benzaldehyde (>99.9% pure - impurity detected was benzoic acid)
Reliability: (2) valid with restrictions
The data was collected prior to GLP by a reliable method and published in a peer-reviewed journal and are consistent with chemical structure.
16-NOV-2001 (1)

2.5 Partition Coefficient

log Pow: = 1.48 at 25 degree C

Method: other (measured)
Year: 1995
GLP: no data

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (16)

log Pow: = 1.71 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (49)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 6570 mg/l at 25 degree C

Method: other
Year: 1992
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.

Solubility in: Water
Value: = 6100 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a log Kow = 1.48
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (51)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 7.2 hour(s)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (46)

3.1.2 Stability in Water

Type: abiotic

Method: other:
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Substance is an aldehyde and will not hydrolyze in water.
16-NOV-2001

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 355000
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.19
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 3.71
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.00011
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.51
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.59
Aerosol =0.000036% Air =5.09% Fish =0.00014% Sediment =0.055% Soil =2.47% Suspended Sediment =0.0017% Water =92.4%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (45)

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 1.1; nonlinear - 1.0. Expert survey results: ultimate - 3 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Benzaldehyde is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (47)

Type: anaerobic
Inoculum: activated sludge
Result: other: Total degradation

Method: other: Chemical oxygen demand
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Degradation % after time: 99% at <120 hours
Kinetic: 119 mg COD/gL
Time required for 10% degradation: <120 hours
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Contact time: Up to 120 hours
Innoculum: From activated sludge
The concentration of test material is increased during activation until it reaches 200 mg/L COD. Degradation is carried out on an initial concentration equivalent to 200 mg/L COD and continues until there is no measured decrease in COD.
Conclusion: Benzaldehyde is classified to be readily degradable.
Reliability: (2) valid with restrictions
The data were obtained prior to GLP and OECD guidelines but data are consistent with chemical structure. Some details are not available but published in a peer reviewed journal.
16-NOV-2001 (36)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Larval survival and growth test
Species: Pimephales promelas (Fish, fresh water)
Unit: **Analytical monitoring:** yes

Method: other: Experimental
Year: 1996
GLP: yes
Test substance: other TS

Result: The NOEC for growth of 7-d larvae was 1.8 mg/L. The NOEC for 1- and 4- day larvae was <0.9 mg/L. NOEC for survival of 1- and 4-d larvae was 3.6 mg/L (first test). NOEC for survival in second test was 0.22 mg/L for 1-d larvae and 1.8 mg/L for 4- and 7-d.
Test condition: Each test concentration had 4 replicates with 10 larvae each. Larvae were fed once on the 1st day and 2x/day on days 1-6. Dead and surviving larvae were counted and survivors were prepared for dry weight determination.
Test substance: Benzaldehyde (reagent grade, 99+% purity)
Conclusion: 1-d larvae more sensitive than older larvae to benzaldehyde.
Reliability: (1) valid without restriction
EPA study.
27-APR-2001 (35)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 96 hour LC50 = 13.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (48)

Species: other: Guppy (Poecilia reticulata)
Exposure period: 14 day(s)
Unit: **Analytical monitoring:** yes

Method: other: Calculated
Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Test condition: Experiments were conducted under semi-static conditions with daily renewal of test solutions. A minimum of 5 concentrations of benzaldehyde were tested. The guppies were acclimated for 12 days prior to testing and 10 guppies/concentration were tested. The control group consisted of 12 guppies exposed to the test substance carrier solvent, acetone. Logit transformation was used to calculate the LC50.
Conclusion: The 14-day LC50 for benzaldehyde was reported to be 1.57 umoles/L. The log P was calculated to be 1.49 (Rekker, 1977).
Reliability: (2) valid with restrictions
Not indicated if conducted under GLP, but results tabulated in a published journal and considered reliable.
12-MAR-2001 (6)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: 24 hour LC50
Species: Daphnia magna (Crustacea)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1977
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test condition: Daphnia magna (30/group, 24 hours old) were maintained in chlorine free tap water saturated with oxygen, pH of 7.7-7.7 and temperature of 20-22 C. The LC50, LC0 and LC100 were determined.
Conclusion: LC50 = 50 mg/L; LC0 = 6.3 mg/L; LC100 = 100 mg/L
Reliability: (2) valid with restrictions
The study was reported in German with an English summary. The results are considered reliable.
28-MAR-2001 (2)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 48 hour LC50 = 12.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (48)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = 152 -

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 96 hour EC50 = 152.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (48)

Species: other aquatic plant: Chlorella vulgaris
Unit: **Analytical monitoring:** no data

Method: other: Growth and cell division
Year: 1971
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Appropriate statistical evaluations: None reported
Control response: Unknown
Remark: Biological observations: Growth inhibition of 30, 30 and 95% after 80 hours and 7, 10 and 90% after 160 hours at 0.00005, 0.0001, and 0.001 M, respectively.
Endpoint basis: Inhibition of cell growth
Test condition: Cell growth was determined by direct cell counting using a Burker counting chamber.
Exposure period: 80 and 160 hours
Nominal concentration: 0.00005, 0.0001, and 0.001 M
Conclusion: In a dose-dependent manner, benzaldehyde inhibited growth of Chlorella vulgaris.
Reliability: (2) valid with restrictions
Published data, reasonably well described. No statistics performed.

14-MAR-2001 (5)

Species: other aquatic plant: Chlorella vulgaris
Exposure period: 5 hour(s)
Unit: **Analytical monitoring:** yes

Method: other: Respiration of photosynthesizing cells
Year: 1971
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Analytical monitoring: Warburg respirometer
Appropriate statistical evaluations: None reported
Control response: Unknown
Remark: Biological observations: Stimulation of respiration of 0, 10, and 90% at pH 5.6 and 5, 50, and 60% at pH 7.2 at 0.00005, 0.0001, and 0.001 M, respectively.
Endpoint basis: Stimulation of cell respiration (oxygen uptake)
Test condition: Cells were maintained in the dark at 25 C in 20 ml flasks and oxygen uptake was measured over a period of 5 hours at a pH of 5.6 or pH of 7.2.
Nominal concentration: 0.00005, 0.0001, and 0.001 M
Conclusion: In a dose-dependent manner, benzaldehyde stimulated the respiration of cells from Chlorella vulgaris.
Reliability: (2) valid with restrictions
Published data, reasonably well described. No statistics performed.

14-MAR-2001 (4)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: other: oral acute toxicity
Species: rat
Strain: other: white
Sex: no data
Vehicle: no data

Method: other: LD50 calculated
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 = 2850 mg/kg bw (no confidence limits reported)
Number of deaths at each dose level: Not reported
Test condition: Not reported
Total of 70 rats orally administered benzaldehyde and observed for 7 days.
Reliability: (4) not assignable
Study translated from foreign article with very limited description.
30-JUN-2001 (43)

Type: LD50
Species: rat
Strain: Osborne-Mendel
Sex: male/female
No. of Animals: 5
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by using the Litchfield and Wilcoxon, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Slope function: 1.4 (95% C.L. 1.2-1.6). Depression and coma were observed at higher doses. Time of deaths was between 4 and 18 hours.
Result: LD50 = 1300 mg/kg bw (95% C.L. 1110-1540)
Number of deaths at each dose level: Not reported
Test condition: Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
30-JUN-2001 (22)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: male/female
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by using the Litchfield and Wilcoxon, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Slope function: 1.4 (95% C.L. 1.2-1.8). Diuresis, tremors, intestinal irritation and haemorrhage were observed. Time of deaths was between 1 hour and 4 days.
Result: LD50 = 1000 mg/kg bw (95% C.L. 800-1250)
Number of deaths at each dose level: Not reported
Test condition: Groups of guinea pigs consisting of both males and females were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Not reported
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (22)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 4
Vehicle: no data

Method: other: LD50 calculated
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Skin irritation was graded as moderate redness and edema at 1.25 g/kg bw.
Result: LD50 = >1.25 g/kg bw
Number of deaths at each dose level: 0/1 and 4/4 deaths at 1250 and 5000 mg/kg bw, respectively.
Test condition: Groups of 1 and 4 rabbits were topically administered 1.25 or 5.0 g/kg bw of test substance, respectively.

Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results. Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (28)

5.1.4 Acute Toxicity, other Routes

Type: other: intraperitoneal acute toxicity
Species: mouse
Strain: other: white
Sex: no data
Vehicle: no data
Route of admin.: other: Intraperitoneal

Method: LD50 calculated
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 = 3265 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: Not reported
Total of 130 mice were intraperitoneally administered benzaldehyde and observed for 7 days.
Reliability: (4) not assignable
Study translated from foreign article with very limited description.
11-MAR-2001 (43)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 16 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 10,000 ppm Actual dose: approximately 500 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 10,000 ppm for 16 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Conclusion: No effect reported at 10,000 ppm benzaldehyde in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (14)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 27-28 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 1,000 ppm Actual dose: approximately 50 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 1,000 ppm for 27-28 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Conclusion: No effect reported at 1,000 ppm benzaldehyde in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (15)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: White
Route of administration: gavage
Exposure period: 8 weeks
Frequency of treatment: alternate days
Post exposure period: None
Doses: 10 mg/rat on alternate days (approximately 20 mg/jkg bw/d) Actual dose: Approximately 20 mg/kg bw/d
Control Group: other: Not described

Method: other: 8-week gavage study
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No hepatic enzyme activity was reported.
Test condition: Benzaldehyde was administered orally to adult white rats at doses of 10 mg diluted in 0.1 ml of oil on alternate days for a period of 8 weeks. Animals were killed and livers were examined for biochemical changes.
Reliability: (4) not assignable
Study translated from foreign article with very limited description.
11-MAR-2001 (43)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: White
Route of administration: gavage
Exposure period: 12 weeks
Frequency of treatment: alternate days
Post exposure period: None
Doses: 10/rat on alternate days (approximately 20 mg/kg bw/d) Actual dose: Approximately 20 mg/kg bw/d
Control Group: other: not described

Method: other: 12-week gavage study
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: There were no effects on growth, liver or adrenal gland weight.
Test condition: Benzaldehyde was administered orally to adult white rats at doses of 10 mg diluted in 0.1 ml of oil on alternate days for a period of 12 weeks. 32 rats were divided into 4 groups, 2 of which were administered benzaldehyde in 2 different diet blends (18 or 8% casein). The other 2 groups were not described.
Reliability: (4) not assignable

Study translated from foreign article with very limited description.

11-MAR-2001 (43)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 16 days
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 100, 200, 400, 800, or 1,600 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: 16-day gavage study
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).
Result: LOAEL: 800 mg/kg bw/d
NOAEL: 400 mg/kg bw/d
Toxic response/effects by dose level: All high dose rats died on day 2. 2 male and 2 female rats died in the 800 mg/kg bw/d dose group. Decreased final body weights (14% in males and 11% in females) were reported in the 800 mg/kg bw/d dose group. No other compound-related effects were reported.
Test condition: Groups of 5 male and 5 female rats were administered 0, 100, 200, 400, 800, or 1,600 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 16 days. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on days 1 and 8 and necropsied at study termination.
Test substance: Benzaldehyde (99% purity)
Conclusion: Based on increased mortality, the NOEL was determined to be 400 mg/kg bw/day.
Reliability: (1) valid without restriction
NTP study

04-MAR-2001 (31)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 50, 100, 200, 400, or 800 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: 13-week gavage study
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.)

Result: LOAEL: 800 mg/kg bw/d
NOAEL: 400 mg/kg bw/d
Toxic response/effects by dose level: At the highest dose, final mean body weight of male rats was 26% lower than controls and 6/10 males and 3/10 females died before the study ended. In addition, multiple histopathological effects were reported including: degeneration and necrosis of the cerebellum, necrosis of the neurons in the hippocampus, hyperplasia and/or hyperkeratosis of the forestomach (with mild to moderate thickening of the squamous epithelium, degeneration of the liver, necrosis of the liver (males only), and degeneration or necrosis of the tubular epithelium in the kidney. No other compound-related effects were reported. One female rat in the 400 mg/kg bw/day group and one female control rat died.

Test condition: Groups of 10 male and 10 female rats were administered 0, 50, 100, 200, 400, or 800 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 13 weeks. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on day 0, once per week and at termination. All animals were necropsied at study termination and complete histopathologic examinations were conducted on controls, and 2 highest dose groups.

Test substance: Benzaldehyde (99% purity)

Conclusion: Based on the various lesions reported at 800 mg/kg bw/d, but not at 400 mg/kg bw/d, doses selected for the 2-yr study were 200 and 400 mg/kg bw/d.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: up to 104 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 200, or 400 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: Carcinogenicity assay
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 400 mg/kg bw/d
NOAEL: 200 mg/kg bw/d
Toxic response/effects by dose level: There was no effect on body weights. Survival was significantly decreased in the high-dose male group after day 373. No other effect on survival was reported. In high-dose males, hyperlasia and adenomas of the exocrine pancreas were marginally increased but the incidence of adenomas was within the range of historical corn oil vehicle controls. There was a marginal increase in the incidence of malignant mesoteliomas of the tunica vaginalis and/or peritoneum in treated male rats; however, since there was no significant increase in high-dose males relative to historical controls, the malignant mesotheliomas were not considered related to benzaldehyde treatment. There was a positive trend for mononuclear cell leukemia in male rats thought to be due to an increase in stage 1 leukemia. The slight increases in mononuclear cell leukemia reported were not considered to be related to benzaldehyde treatment. In 2 females at the highest dose, squamous papillomas were reported but due to lack of accompanying hyperplasia and comp

Test condition: Groups of 50 male and 50 female rats were administered 0, 200, or 400 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 2 years. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on day 0, once per week for the first 13 weeks, once per month for the remainder of the study and at termination. All animals were necropsied at study termination and complete histopathologic examinations were conducted on controls, highest dose group, and on low-dose animals dying before study end.

Test substance: Benzaldehyde (99% purity)

Conclusion: Under the conditions of the 2-yr gavage study, there was no evidence of carcinogenic activity of benzaldehyde for male or female F344/N rats receiving 200 or 400 mg/kg bw/d.

Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (31)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Doses: 0, 50, 100, 200, 400, or 800 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 13-week toxicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: NOAEL: 400 mg/kg bw/d
Toxic response/effects by dose level: Benzyl alcohol decreased survival (8 males and 2 females died; half of the deaths were a result of gavage error) and body weight (7% in males and 5% in females) at the highest dose tested (800 mg/kg bw/d). High-dose rats staggered, were lethargic, and had labored breathing. Five male rats also had blood around the nose and mouth. In addition, histopathologic examination of high-dose rats revealed lesions in the brain (necrosis of the dentate gyrus of the hippocampus), skeletal muscle (necrosis-males only), thymus (congestion, hemorrhage and atrophy-males only) and kidney (nephrosis-males only). The renal lesions were non-specific and similar to age-related renal disease.

Test condition: Groups of 10 male and 10 female rats were administered 0, 50, 100, 200, 400, or 800 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 13 weeks. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week, and necropsied at study termination.

Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)

Conclusion: No adverse effects were produced at 400 mg/kg bw/d or lower.

Reliability: (1) valid without restriction
NTP study
10-JUL-2001 (30)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: daily, 5 days/week
Doses: 0, 200, or 400 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 2-year carcinogenicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.
The increase in the incidence of hemorrhage and foreign material in the lung of treated animals may have been related to the gavage procedure particularly since there was a high rate of gavage-related deaths (50% in females).

Result: Toxic response/effects by dose level: Body weights were comparable to controls and no compound-related clinical signs were reported. Sialodacryoadenitis was diagnosed in all groups. Survival was significantly decreased in high-dose females after week 50 (p=0.013) and in low-dose females after week 71 (p=0.059). High-dose rats showed an increased incidence of cataracts and retinal atrophy considered to be due to exposure to fluorescent light since the cages were housed on the top 2 rows until the last 10 weeks of the study. In 4 high-dose males, epithelial hyperplasia of the forestomach was observed. 1/19 low-dose and 1/50 high-dose male rats had a squamous cell papilloma. In the lung, there was an increase in the incidence of hemorrhage and foreign material in treated rats dying before study termination. In males, this was dose-related and included acute inflammation in the nasal tract, hemorrhage in the larynx, and edema in the lungs. In females, the incidence of anterior pituitary gland neoplasms (adenomas and combined adenomas or carcinomas) decreased w

Test condition: Groups of 50 male and 50 female rats were administered 0, 200, or 400 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 103 weeks. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week for the first 13 weeks and monthly thereafter. All animals were necropsied at study termination and complete histopatholic examinations were conducted on controls, highest dose group, and on low-dose animals dying through month 21 of study.

Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)

Conclusion: The NTP concluded that benzyl alcohol produced “no evidence of carcinogenic activity” in this study.

Reliability: (1) valid without restriction

NTP study
10-JUL-2001 (30)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 16 days
Frequency of treatment: daily, 5 days/week
Doses: 0, 125, 250, 500, 1,000 or 2,000 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 16-day toxicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).
Result: NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: The 125 mg/kg bw/d dose was prepared at 10-fold too high on days 8 and 9. All high dose animals died by day 2. Two male and 3 female rats died in the 1,000 mg/kg bw/d group. Decreased final mean body weight (18% in males) was reported in the 1,000 mg/kg bw/d group. At the 2 highest doses, lethargy was reported and rats had blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts. Rough coats were reported at 250 mg/kg bw/d in females, 500 mg/kg bw/d in both sexes, and 1,000 mg/kg bw/d in males. No histological findings were reported.
Test condition: Groups of 5 male and 5 female rats were administered 0, 125, 250, 500, 1,000 or 2,000 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 16 days. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week, and necropsied at study termination.
Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)
Conclusion: Based on increased mortality, the NOAEL was determined to be 500 mg/kg bw/d.
Reliability: (1) valid without restriction
NTP study
10-JUL-2001 (30)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: other: inhalation
Exposure period: 14 days
Frequency of treatment: 6 hours/day
Post exposure period: up to 72 hours
Doses: 500, 750, or 1,000 ppm
Control Group: other: filtered air

Method: other: 14-day inhalation study
Year: 1991
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results analyzed using Duncan's multiple range test and a modified least significant difference procedure. Differences between groups determined with Student's t-test.
The authors suggested that the reported goblet cell metaplasia corresponded to a “mild form of adaptation during the recovery period following the inhalation exposure to benzaldehyde”.

Result: LOAEL: 500 ppm
Toxic response/effects by dose level: In the first week, 10 females and 1 male from the 1,000 ppm group and 1 female from the 750 ppm group died. Two more females were moribund in the second week. At all exposure groups, a mild irritation of the mucosa was reported with effects on the central nervous system including tremors, hypothermia, reduced breathing rates and decreased motor activity. At 1,000 ppm, hemoglobin and hematocrit counts were decreased in both sexes. Red blood cells also were decreased in high-dose females. A dose-related increase in monocytes was observed in females. Also in females, significant changes in total protein, albumin fraction, and serum cholinesterase were reported. Aspartate aminotransferase was significantly increased at all exposures in both males and females. In males, goblet cell metaplasia (largely confined to the respiratory epithelium lining the nasal septum) was reported; however the incidence and severity were similar in all treatment groups. There was no change in females.

Test condition: Groups of 14 male and 14 female Sprague-Dawley rats were exposed to 500, 750, or 1,000 ppm benzaldehyde, 6 hours/day for 14 consecutive days in 2.5 m3 chambers with an air flow rate of 500 L/min (Laham et al., 1991). Body weights were recorded after day 2, 8 and 14. At necropsy, blood samples were collected for hematological and biochemical analyses, and gross pathological and histopathological examinations were conducted.

Test substance: Benzaldehyde (98% purity)

Conclusion: A NOAEL could not be determined, but the authors concluded that typical human exposure levels are so small (5.3-22.5 ppb) that they should not be considered hazardous.

Reliability: (1) valid without restriction
Although it was not reported whether this study was conducted under GLP, it was well reported and conducted by the Canadian Health Protection Branch. The data are considered reliable.
11-MAR-2001 (24)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: other: White
Route of administration: gavage
Exposure period: 3 months
Frequency of treatment: daily
Post exposure period: Not reported
Doses: 80 mg/kg bw/d
Control Group: other: not reported

Method: other: 3-month gavage study
Year: 1970
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
The reduced body weight gain was reported not to be due to reduced feed intake, but it may have been due to stress factors such as food restriction, low temperature, swimming test, centrifugation, carbon tetrachloride detoxication test or kidney function testing.
Result: Toxic response/effects by dose level: Weight gain of the treated animals was reduced compared to control animals.
Test condition: Groups of 50 male and 50 female crossbred white mice (strain not specified) were administered 80 mg benzoic acid/kg bw/d by oral intubation for 3 months.
Test substance: Benzaldehyde (data for structurally related benzoic acid)
Reliability: (4) not assignable
Very limited description of study and results.
12-MAR-2001 (41)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 16 days
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 200, 400, 800, 1,600, or 3,200 mg/kg bw/d
Control Group: other: yes-vehicle

Method: other: 16-day gavage study
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).

Result: LOAEL: 800 mg/kg bw/d
NOAEL: 400 mg/kg bw/d
Toxic response/effects by dose level: All mice in the 2 highest dose groups died by day 3. One male receiving 800 mg/kg bw/d died on day 10. No other compound related effects were reported.

Test condition: Groups of 5 male and 5 female mice were administered 0, 200, 400, 800, 1,600, or 3,200 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 16 days. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on days 1 and 8 and necropsed at study termination.

Test substance: Benzaldehyde (99% purity)

Conclusion: Based on increased mortality, the NOEL was determined to be 400 mg/kg bw/day.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 75, 150, 300, 600, or1,200 mg/kg bw/d
Control Group: other: yes-vehicle

Method: other: 13-week gavage study
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 600 mg/kg bw/d
NOAEL: 300 mg/kg bw/d
Toxic response/effects by dose level: At the highest dose, 9/10 males and 1/10 females died. At 600 mg/kg bw/d, the final mean body weight of males was 9% lower than controls. Mild to moderate renal tubule degeneration occurred in all males in the high-dose group and in 1/10 males in the 600 mg/kg bw/d group. No other compound related effects were reported.

Test condition: Groups of 10 male and 10 female mice were administered0, 75, 150, 300, 600, or 1,200 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 13 weeks. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on day 0, once per week, and at the end of the study. All animals were necropsied at study termination and complete histopathologic examinations were conducted on controls, and 2 highest dose groups.

Test substance: Benzaldehyde (99% purity)

Conclusion: Based on the mild renal lesions and depressed body weight gain, the doses selected for the 2-yr study were 300 and 600 mg/kg bw/d.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: daily 5 days/week
Post exposure period: No
Doses: 0, 300, or 600 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: Carcinogenicity assay
Year: 1990
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: Toxic response/effects by dose level: There was no effect on body weight gain or survival. Focal hyperplasia characterized by increased thickness of the stratified squamous epithelium and squamous cell papillomas of the forestomach (male: control 1/50; low dose, 2/50; high dose, 5/50; female: control, 0/50; low dose, 5/50; high dose 6/50) were increased in treated animals.

Test condition: Groups of 50 male and 50 female mice were administered 0, 300, or 600 mg benzaldehyde/kg bw/d in corn oil by gavage, 5 days/week for a period of 103 weeks. Animals were housed 5 per cage. Water and feed were available ad libitum. Animals were observed 2X per day, weighed on day 0, once per week for the first 13 weeks, once per month for the remainder of the study and at termination. All animals were necropsied at study termination and complete histopathologic examinations were conducted on controls, highest dose group, and on low-dose animals dying before study end.

Test substance: Benzaldehyde (99% purity)

Conclusion: Under the condiitions of the 2-yr gavage study, there was some evidence fo carcinogenic activity of benzaldehyde for male or female B6C3F1 mice, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 16 days
Frequency of treatment: daily, 5 days/week
Doses: 0, 125, 250, 500, 1,000 or 2,000 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 16-day toxicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).
Result: NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: The 125 mg/kg bw/d dose was prepared at 10-fold too high on days 8 and 9. All high dose animals died by day 1. One male and 2 female mice died in the 1,000 mg/kg bw/d group. Llethargy and rough coat were reported in males at the 3 highest doses and in females at the 2 highest doses. No histological findings were reported.
Test condition: Groups of 5 male and 5 female mice were administered 0, 125, 250, 500, 1,000 or 2,000 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 16 days. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week, and necropsied at study termination.
Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)
Conclusion: Based on increased mortality, the NOAEL was determined to be 500 mg/kg bw/d.
Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Doses: 0, 50, 100, 200, 400, or 800 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 13-week toxicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: NOAEL: 400 mg/kg bw/d (m); 200 mg/kg bw/d (f)
Toxic response/effects by dose level: Deaths occurred in all treatment groups, but with the exception of one high-dose female, all were judged to be due to gavage error. Benzyl alcohol produced slight decreases in body weight of 5 and 8% in females at 400 and 800 mg/kg bw/d, respectively. At the highest dose level, staggering was seen in both male and female mice during the first 2 weeks of the study. All groups were reported to show chronic interstitial pneumonia related to a Sendai infection. No compound-related histopathology was reported.

Test condition: Groups of 10 male and 10 female mice were administered 0, 50, 100, 200, 400, or 800 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 13 weeks. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week, and necropsied at study termination.

Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)

Conclusion: No adverse effects were produced at 200 mg/kg bw/d or lower in females and 400 mg/kg bw/d or lower in males.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: daily, 5 days/week
Doses: 0, 100, or 200 mg/kg bw/d
Control Group: other: corn oil control (vehicle)

Method: other: 2-year carcinogenicity study
Year: 1989
GLP: yes
Test substance: other TS

Remark: During week 80 for a period of 4 days, the mice were accidently administered 375 or 750 mg alpha-methyl benzyl alcohol/kg bw/d instead of 100 or 200 mg benzyl alcohol/kg bw/d. This error did not appear to cause any adverse effects. The increased incidence of corpora amylacea in the brain was not statistically significant and is a common, spontaneously occurring lesion. Although there was a slight increase in the incidence of adrenal cortex adenomas in high-dose males, the NTP considered the incidence to be within the historical range and not compound related. The increased incidence of lung congestion and foreign material may have been due to the gavage procedure.
Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: Toxic response/effects by dose level: Body weights were comparable to controls and no compound-related clinical signs were reported. After week 74, the survival of control females was significantly decreased compared to the high-dose females. No other effects on survival were noted. In the brain, an non-statistically significant increased incidence of corpora amylacea was reported in high-dose animals (in males, 15/49, 21/48, and 22/50 and in females, 14/50, 15/48, and 25/50 for control, 100 and 200 mg/kg bw/d groups, respectively). Male mice in the high-dose group showed a slight increase (P=0.044) in the incidence of adrenal cortex adenomas. In males, the incidence of Harderian gland adenomas tended to decrease with increasing dose. Also, lung congestion occurred with a non-statistically significant increased incidence in high-dose males. Similarly, the incidence of foreign material in the lung was increased in low-dose mice.

Test condition: Groups of 50 male and 50 female mice were administered 0, 100, or 200 mg benzyl alcohol/kg bw/d in corn oil by gavage, 5 days/week for a period of 103 weeks. Animals were housed 5 per cage and water and feed were available ad libitum. Animals were observed 2X per day, weighed 1X/week for the first 13 weeks and monthly thereafter. All animals were necropsied at study termination and complete histopatholic

examinations were conducted on controls, highest dose group, and on low-dose animals dying through month 21 of study.
Test substance: Benzaldehyde (data for metabolic precusor, benzyl alcohol)
Conclusion: The NTP concluded that benzyl alcohol produced “no evidence of carcinogenic activity” in this study.
Reliability: (1) valid without restriction
NTP study
10-JUL-2001 (30)

5.5 Genetic Toxicity 'in Vitro'

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 5-160 ug/ml (without S9); 160-1600 ug/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Sister chromatid exchange (Galloway et al., 1985)
Linear regression analysis was used to test trend. A 20% absolute increase over the control, at each dose, was considered to be significant.
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The induction of sister chromatid exchanges was positive without S9 and weakly positive with S9.
Result: Benzaldehyde induced sister chromatid exchanges with and without metabolic activation.
Test condition: Chemical treatment periods were approximately 25 hours without S9 (after 2 hours of test chemical exposure, 5-bromodeoxyuridine was added) and 2 hours with S9 (after which 5-bromodexoyuridine was added). After treatment with hypotonic KCl, cells were fixed, stained and examined with fluorescent microscopy. 50 cells per dose were scored from the three highest concentrations when sufficient M2 cells were available, from the control groups..
Metabolic activation: with and without rat liver microsome fraction S9 and cofactors
Conclusion: Benzaldehyde was an inducer of sister chromatid exchanges, but the effect was weak in the presence of metabolic activation.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
01-APR-2001 (13)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA102, TA104
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay The data were evaluated using Wahrendorf ranking and Dunnett's t-test.
Year: 1992
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde was non mutagenic with or without S9 in all Salmonella strains tested.
Test condition: Cells were tested with and without S9.
Metabolic activation: rat liver microsome fraction S9 from Aroclor induced F344 rats and B6C3F1 mice
Conclusion: Benzaldehyde was non mutagenic.
Reliability: (2) valid with restrictions
Data from abstract only but method appears to be standard and results are consistent with chemical structure and other results.
15-MAY-2001 (8)

Type: Mouse lymphoma assay
System of testing: non bacterial L5178Y mouse lymphoma cell line
Concentration: Trial #1: 0, 50, 100, 200, 400 or 800 ug/ml; Trial #2: 0, 80, 160, 320, 480, or 640 ug/ml
Cytotoxic Concentration: 640 ug/ml
Metabolic activation: with
Result: positive

Method: other: Mouse lymphoma assay The data were evaluated using the dose-trend test (Barlow et al., 1972) and a variance of analysis of pair-wise comparisons.
Year: 1991
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde significantly increased mutant fractions in both experiments without S9 but at concentrations close to cytotoxic levels. The lowest-observed-effective dose (LOED) was 400 ug/ml and concentrations of 640 ug/ml were lethal.
Test condition: DMSO was used as the solvent and control (4 cultures). Methanesulphonate at 15 ug/ml was used as the positive control (2 cultures). Four cultures for each concentration were prepared. Colonies were counted using an automated counter. If relative growth and/or cloning efficiency did not meet the predetermined quality control criteria, then the culture was rejected.
Metabolic activation: rat liver microsome fraction S9
Conclusion: Benzaldehyde increased mutant fractions but only at

concentrations nearing lethal levels.
Reliability: (1) valid without restriction
NTP study
01-APR-2001 (26)

Type: other: clastogenic assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 50 nM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Chromsomal aberrations Chi square test
Year: 1982
GLP: no
Test substance: other TS

Result: Benzaldehyde produced a significant difference in chromosomal aberrations compared to the vehicle control ($p<0.001$).
Test condition: DMSO was used as the solvent and control. Cells were exposed to the flavoring agent for 24 hours and then incubated another 24 hours without the flavor after which the cells were treated with colchicine for 2-3 hours. Cells were stained using Giemsa staining method. The scoring of about 200 metaphase spreads, containing 20-26 chromosomes was used to calculate the percentage of chromosomal aberrations. Metabolic activation: rat liver microsome fraction S9
Test substance: Benzaldehyde (90-95% purity)
Conclusion: Benzaldehyde induced chromosomal aberrations.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.
02-APR-2001 (23)

Type: Sister chromatid exchange assay
System of testing: non bacterial Human lymphocytes
Concentration: 0-2.0 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange (Jansson et al., 1986) The data were analyzed using linear regression by least squares and significance was tested at $p<0.05$, 0.01, and 0.001.
Year: 1988
GLP: no
Test substance: other TS

Result: Statisticially significant increase in sister chromatid exchanges ($p<0.01$) as compared to the vehicle control. The regression coefficient was 4.0 SCE/cell/mM.
Test condition: DMSO and ethanol were used as solvents and negative controls. The positive control used was styrene-7,8-oxide.

After an exposure of 88 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified), 25 metaphases from one culture were analysed.
Metabolic activation: Phytohemagglutinin-stimulated

Test substance: Benzaldehyde (98% purity)
Conclusion: Benzaldehyde induced sister chromatid exchanges in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

04-JAN-2001 (21)

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster K-1 ovary cells (ATCC)
Concentration: 0, 3.3, 10, 33.3, 100, 333, or 1000 uM
Cytotoxic Concentration: 1000 uM
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange Student's t-test
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde did not induce sister chromatid exchanges at concentrations up to 333 uM. The highest concentration was reported to be cytotoxic.
Test condition: Chinese hamster ovary cells were exposed to mitomycin C for 21 hours and then cells were exposed to benzaldehyde for 1 cell cycle followed by addition of 5-bromodeoxyuridine 2 cell cycles prior to fixation. Fifty metaphases per culture were analyzed for sister chromatid exchanges with and without treatment with mitomycin C for SCE induction.
Metabolic activation: mitomycin C (induction of SCEs)
Conclusion: Benzaldehyde was not an inducer of sister chromatid exchanges in this assay.
Reliability: (2) valid with restrictions
Published in peer review journal but limited description of protocol and tabulated results.

30-MAR-2001 (40)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA98, TA100
Concentration: 3 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Spot tests are less sensitive than quantitative experiments. Also, the results from strain TA100 are difficult to interpret because the high growth background (150-200 colonies per plate); however, spot tests provide a good "screening" method for large numbers of chemicals.
Result: Benzaldehyde produced a negative response in this assay.
Test condition: For each experiment viable count was determined, the number of spontaneous revertants was measured, the presence of the rfa-mutation was determined by crystal violet inhibition, the presence of the plasmid pKM 101 in strains TA98 and TA100 was determined by resistance to ampicillin, and the response to positive controls N-methyl-N-nitrosoquanidin (without metabolic activation) and 2-aminoanthracene (with activation) was determined. Spectroscopic-grade ethanol was used as the solvent. The test substance was tested at 3 umol/plate in TA98, TA100, TA1535, and TA1537 with or without S9. If there was no background lawn of bacteria, the tests were redone using lower concentrations. Uncertain results prompted the conduction of the tests at 4 concentration levels (0.03, 0.3, 3 and 30 umol/plate). Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats
Conclusion: Benzaldehyde is non-mutagenic in the Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 with or without S9.
Reliability: (1) valid without restriction
Study is published in a peer reviewed journal with adequate description and follows standard procedures.

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA1535, TA1537, and TA98
Concentration: 0, 100, 333, 1,000, 3,333, 5,000 or 6,666 ug/plate for all tester strains
Cytotoxic Concentration: 3,333 ug/plate for TA100, 6,666 ug/plate for TA1535, TA1537, and TA98
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1989
GLP: yes
Test substance: other TS

Result: Benzyl alcohol produced negative results in all tester strains.
Test condition: Benzyl alcohol was tested in duplicate with and without metabolic activiation. Positive controls used were 2-aminoanthracene, 4-nitro-o-phenylenediamine, sodium azide, and 9-aminoacridine.
Metabolic activation: S9 from Aroclor 1254-induced male Syrian hamster or male SD rat liver
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was non mutagenic in this assay.
Reliability: (1) valid without restriction
NTP study
08-JUL-2001 (32)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Concentration: 37500 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay Not reported
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde was inactive in the Ames assay using Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100 with or without S9 activation.
Test condition: Following 2 days of incubation at 37 C, revertant colonies were counted electronically.
Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats
Conclusion: Benzaldehyde was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.

30-MAR-2001 (18)

Type: Unscheduled DNA synthesis
System of testing: rat hepatocyte Rat hepatocyte (Fischer and Sprague-Dawley)
Concentration: 251 ug/ml
Cytotoxic Concentration: not reported
Metabolic activation: no data
Result: positive

Method: other: Unscheduled DNA synthesis (Williams, 1977, 1980 and Butterworth et al., 1987) Not reported
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde treatment did not increase UDS compared to controls.
Test condition: Rat hepatocytes were incubated in culture dishes for 18-20 hours with benzaldehyde. Concurrent cell counting or measurement of LDH release was used to determine relative cell survival. UDS was measured by electronically counting nuclear grains and calculating the net nuclear grain count (NNG). At each test concentration, 75-150 cells were analyzed. An increase in NNG of "at least 6 grains per nucleus above the concurrent solvent control value and/or an increase in the percent of nuclei having 6 or more net grains to at least 10% above the concurrent negative control" was considered a positive UDS response.
Conclusion: Benzaldehyde was not genotoxic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.

01-APR-2001 (19)

Type: Mouse lymphoma assay
System of testing: non bacterial L5178Y mouse lymphoma cell line
Concentration: 12.5-800 nl /ml (with and without S9), 25-600 nl/ml (without S9), 400-600 nl/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: negative

Method: other: Mouse lymphoma assay (Clive et al., 1979) Not reported
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: No increase in mutagenesis (as compared to the negative controls) except at 400-600 nl/ml (with S9) where a 2.8-5.2 fold increase was observed.
Test condition: Cells were exposed to benzaldehyde for 4 hours, washed, incubated for 48 hours and then cloned. After 10-14 days,

colonies were automatically counted. The ratio of mutant to viable colonies cloned without selective medium was considered to be the mutant frequency.
Metabolic activation: with and without rat liver microsome fraction S9 and cofactors

Conclusion: Although benzaldehye produced an increase in mutagenic activity at concentrations ranging from 400-600 nl/ml (with S9), no change in mutagenic activity was reported at the other concentration ranges (12.5-800 and 25-600 nl/ml) that also cover the range reportedly showing an effect. Without further detail regarding the study design, it is difficult to interpret the significance of the positive finding.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.

01-APR-2001 (19)

Type: Chromosomal aberration test
System of testing: Chinese hamster ovary cells Chinese hamster ovary cells
Concentration: 50-500 ug/ml (without S9); 160-1600 ug/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: negative

Method: other: Chromosomal aberrations (Galloway et al., 1985) Linear regression analysis, binomial sampling assumption, and Dunnett's method for multiple dose comparison were used to evaluate the data.
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Benzaldehyde was negative for chromosomal aberration induction.
Result: No aberration induction was observed.
Test condition: Metabolic activation: with and without rat liver microsome fraction S9 and cofactors
Positive controls consisted of treatment with mitomycin C, triethylenemelamine, or cyclophosphamide and negative controls were solvents used to dissolve the test chemical. Tests were carried out with (2-hr test substance exposure) or without S9 (exposure throughout incubation) activation (male Sprague-Dawley rat hepatocytes induced with Aroclor 1254). Cells were harvested 8-12 hours after the beginning of the treatment, yielding cells in mitosis. 100 cells were scored from each of the three highest dose groups having sufficient metaphases for analysis and from positive and solvent controls. All types of aberrations were recorded and they were grouped as either "simple", "complex", or "other" and "total".
Conclusion: Benzaldehyde did not induce chromosomal aberrations in this assay.
Reliability: (1) valid without restriction

Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (13)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: urinary metabolites assayed ranged from 0.05 to 100 ul/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The urinary metabolites of benzaldehyde were non mutagenic in this assay.
Result: The urinary metabolites of benzaldehyde did not increase the number of revertants.
Test condition: 0.5 ml benzaldehyde was administered by gavage to 2 Sprague-Dawley rats which were kept in metabolism cages. Urine and feces were collected for 24 hours. Urine was separated from the feces and was prepared for mutagenicity screening. Bacterial cells were incubated with benzaldehyde in the presence of S9 for 48 hours at 37 C. Sodium azide and picrolonic acid were used as positive controls for TA100 and TA98, respectively. Enzyme activation by S9 was confirmed with plates containing aflatoxin B1. Metabolic activation: with rat liver microsome fraction S9
Reliability: (2) valid with restrictions
Limited description of study and results.

04-APR-2001 (38)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA102, TA104
Concentration: 33-3333 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay Dunnett's t-test, Wahrendorf ranking and linear regression
Year: 1998
GLP: no
Test substance: other TS

Result: Benzaldehyde produced no increase in reverse mutations.
Test condition: Metabolic activation: with and without rat liver microsome fraction S9 (from Aroclor 1254-induced F344 rats and male B6C3F1 mice)
Test substance: Benzaldehyde (72-91% purity)
Conclusion: Benzaldehyde was non mutagenic with or without metabolic

activation.
Reliability: (1) valid without restriction
Assay was conducted using standard methodology and was published in a peer reviewed journal.
02-APR-2001 (7)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 0.05-500 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1982
GLP: no
Test substance: other TS

Result: Benzaldehyde did not increase the incidence of mutation as compared to the vehicle controls, either with or without S9 mix.
Test condition: DMSO was used as the solvent and control. The results were considered positive if a reproducible, dose-related increase in the number of revertants and a greater than 2-fold increase in spontaneous mutation rate was observed. Metabolic activation: rat liver microsome fraction S9 from Aroclor induced rats
Test substance: Benzaldehyde (90-95% purity)
Conclusion: Benzaldehyde was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.
02-APR-2001 (23)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster cells
Concentration: 0, 0.8, or 1.0 mg/ml without S9; 0, 0.8, 1.0, 1.2 mg/ml with S9
Metabolic activation: with
Result: positive

Method: other: Chromosomal aberrations
Year: 1985
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: At 0, 0.8 and 1.0 mg/ml without S9, the percent polyploid was 0, 11 and 3 and the frequency of total aberrant cells was 2, 2, and 24%, respectively. At 0, 0.8, 1.0, and 1.2 mg/ml with S9, the percent polyploid was 0, 11, 5, and 0 and the frequency of total aberrant cells was 0, 2, 9, and 9%, respectively.
Test condition: Metabolic activation: S9 mixture (not described)
The solvent used was DMSO and the tests were conducted with

and without S9. The percent of polyploid cells was reported, as was the frequency of aberrant cells.

Conclusion: Benzaldehyde was judged by the authors to be positive only without metabolic activation at the highest dose tested.

Reliability: (2) valid with restrictions
Although the data were presented in Japanese with English summary tables, the data were generated by a reputable group of researchers and it appeared that standard procedures were followed. The data were considered reliable.

08-JUL-2001 (42)

Type: Ames test
System of testing: bacterial Salmonella typhimuriumTA98 and TA1535
Concentration: 0, 0.1, 0.5, 1.0, 2.5, or 5.0 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay Mean of 3 values with extremes never removed from the means by >5-10%.
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: There was no detectable mutagenic activity.
Test condition: Metabolic activation: not reported
The test substance was tested at 5 concentrations with 3 plates per concentration. The positive control for TA1535 was 2 ug sodium azide and for TA98 was 3 ug 2-nitrofluorene.

Conclusion: Benzaldehyde was non-mutagenic in this assay.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

03-JUL-2001 (53)

Type: Mouse lymphoma assay
System of testing: non bacterial Mouse L5178Y lymphoma cell
Concentration: Trial 1: 156.25, 312.5, 625, 1,250, 2,500 or 5,000 ug/ml (-S9); Trial 2: 2,500, 3,000, 3,500, 4,000, 4,500, or 5,000 ug/ml (-S9); Trial 3: 250, 500, 1,500, 2,500, or 3,500 ug/ml (with and without S9)
Cytotoxic Concentration: Trials 1&2: 5,000 ug/ml; Trial 3: 3,500 ug/ml
Metabolic activation: with
Result: positive

Method: other: Mouse lymphoma assay (Clive et al. 1979) Not reported
Year: 1989
GLP: yes
Test substance: other TS

Result: Trial 1: cloning efficiency was 62, 88.5, 70, 96, 84, 88 and 85%, Tft-resistant cells was 90.7, 91.5, 121, 165, 140, 175.5 and 384, and mutant fraction was 50.3, 34.5, 57.5, 58, 57, 67 and 150 for solvent control, 156.25, 312.5, 625,

1,250, 2,500 and 5,000 ug/ml. Trial 2: cloning efficiency was 77.5, 84.5, 74, 83.5, 49.5 and 90%, Tft-resistant cells was 87.8, 94.5, 94.5, 107.5, 90, and 282.5, and mutant fraction was 38, 37.5, 43, 43, 58, and 106 for solvent control, 2,500, 3,000, 3,500, 4,000, and 4,500 ug/ml, respectively [high dose was lethal]. Trial 3: cloning efficiency was 83.5, 74, 83.5, 63.5, and 83.5%, Tft-resistant cells was 116.5, 86.5, 100.5, 63, and 88, and mutant fraction was 47.8, 40, 40.5, 32.5, and 35 for solvent control, 250, 500, 1,500, and 2,500 ug/ml, respectively [-S9, high dose was lethal] and cloning efficiency was 64.8, 68, 55.5, 65, and 66%, Tft-resistant cells was 56, 75, 53.5, 70, and 47, and mutant fraction was 29.3, 36.5, 33, 36.5, and 23.5 for solvent control, 250, 500, 1,500, and 2,500 ug/ml, respectively [+S9, high dose was l

Test condition: DMSO was used as the solvent control and mehtyl methanesulfonate was the positive control. All concentrations were tested in duplicate except in trial 1 at the high dose because lethality was seen in one set. Cells were treated for 4 hours, washed, resuspended and incubated for 48 hours. Afterwards, cells were plated with trifluorothymidine (Tft) for selection of Tft-resistant cells. To determine cloning efficiency, 600 cells were plated in nonselective medium. Mutant fraction (ratio of Tft-resistant cells to cloning efficiency divided by 3) was calculated and considered positive if the relative mutant fraction (MF of treated/MF of solvent) was greater than or equal to 1.6.
Metabolic activation: S9 from Aroclor 1254-induced F344 rat liver

Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)

Conclusion: In the absence of metabolic activation and only at cytotoxic levels, benzyl alcohol elevated the frequency of resistant colonies.

Reliability: (1) valid without restriction

08-JUL-2001 (29)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA102, TA104, TA100, TA98, TA1535, and TA1537
Concentration: TA102 (trial 1 and 2): 0, 33, 100, 333, 1,000 or 3,333 ug/plate; TA100 (trial 1): 0, 33, 100, 333, 1,000, or 3,333 ug/plate; TA100 (trial 2): 0, 10, 33, 100, 333, or 1,000 ug/plate; TA1535, TA1537 and TA98: 0, 10, 33, 100, 333, or 1,000 ug/
Cytotoxic Concentration: 1,000 to 3,333 ug/plate
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1990
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: All tester strains produced negative results. Cytotoxicity was observed at 1,000 and 3,333 ug/plate for TA100, at 3,333 ug/plate for TA102 and TA104, and at 1,000 ug/plate for TA98, TA1535 and TA1537.
Test condition: Metabolic activation: S9 mix from livers of Aroclor 1254-incude male SD rat or Syrian hamster
Tester strains and test substance or solvent were incubated with or without S9. Cytotoxicity limited highest concentration tested, but test concentration did not exceed 10 mg/plate. Positive controls used were 2-aminoanthracene, 4-nitro-o-phenylenediamine, sodium azide, and 9-aminoacridine. Tests with TA102, TA104 and TA100 were conducted at Inveresk Research International and with TA1535, TA1537 and TA98 at EG&G Mason Research Institute.
Conclusion: Benzaldehyde was non-mutagenic in this assay.
Reliability: (1) valid without restriction
NTP study
04-JUL-2001 (31)

Type: Ames test
System of testing: bacterial Salmonella typhimuriumTA98 and TA1535
Concentration: 0, 0.1, 0.5, 1.0, 2.5, or 5.0 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay Mean of 3 values with extremes never removed from the means by >5-10%.
Year: 1983
GLP: no
Test substance: other TS

Result: There was no detectable mutagenic activity.
Test condition: Metabolic activation: not reported
The test substance was tested at 5 concentrations with 3 plates per concentration. The positive control for TA1535 was 2 ug sodium azide and for TA98 was 3 ug 2-nitrofluorene.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was non-mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
03-JUL-2001 (52)

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster ovary cell
Concentration: Without S9: Trial 1: 16, 50, 160, 500, or 1,600 ug/ml; Trial 2: 500, 750, 1,000, 1,250, or 1,500 ug/ml; With S9: Trial 1: 16, 50, 160, 500, 1,600, or 5,000 ug/ml; Trial 2: 500, 1,600, 3,000, 4,000 or 5,000 ug/ml
Cytotoxic Concentration: 1,500 ug/ml (-S9); 5,000 ug/ml (+S9)
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange (Galloway et al., 1985)
Year: 1989
GLP: yes
Test substance: other TS

Result: Trial 1(-S9): relative SCEs/cell was 101.1, 110.1, 104.5 and 107.9% for 16, 50, 160, and 500 ug/ml [high dose was lethal]. Trial 2 (+S9): relative SCEs/cell was 111.4, 114.8, 114.8 and 129.5% for 500, 750, 1,000, and 1,250 ug/ml [high dose was lethal]. Trial 1 (+S9): relative SCEs/cell was 97.5, 115.2, 110.1, 112.7 and 115.2% for 16, 50, 160, 500, and 1,600 ug/ml [high dose was lethal]. Trial 2 (+S9): relative SCEs/cell was 103.5, 110.5, 110.5 and 123.3% for 500, 1,600, 3,000, and 4,000 ug/ml [high dose was lethal].
Test condition: Metabolic activation: S9 from Aroclor 1254-induced male SD rat liver
Treated cells were incubated 2 hours with or without S9, then BrdU was added and cells were incubated another 24 (-S9) or 26 (+S9) hours in the presence of colcemid for the last 2-3 hours. DMSO was used as the solvent control and Mitomycin C was the positive control.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol produced a weak, dose-related increase in SCEs both with and without metabolic activation in one of 2 trials. NTP interpreted these results as equivocal because the weak positive response was not reproducible.
Reliability: (1) valid without restriction
NTP study
08-JUL-2001 (29)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster ovary cell
Concentration: Trial 1: 160, 500, 1,600 or 5,000 ug/ml (-S9, 12.5 hrs) and 50, 160, 500, 1,600, or 5,000 ug/ml (+S9, 12.0 hrs); Trial 2: 2,000, 3,000, 4,000, or 5,000 ug/ml (-S9, 12.0 hrs) and 500, 1,600, 3,000, or 4,000 ug/ml (+S9, 15.0 hrs); Trial 3: 25
Cytotoxic Concentration: 3,000 ug/ml (-S9); 5,000 ug/ml (+S9)
Metabolic activation: with
Result: positive

Method: other: Chromosomal aberrations (Galloway et al., 1985)
Year: 1989
GLP: yes

Test substance: other TS

Result: Trial 1: percent cells with aberrations was 3, 2, 3, 2, and 2 for solvent control, 160, 500, 1,600 and 5,000 ug/ml (-S9, 12.5 hrs) and 1, 1, 2, 4, 2, and 2 for solvent control, 50, 160, 500, 1,600, and 5,000 ug/ml (+S9, 12.0 hrs); Trial 2: percent cells with aberrations was 3, 4, 4, 12, and 20 for solvent control, 2,000, 3,000, 4,000, and 5,000 ug/ml (-S9, 12.0 hrs) and 1, 1, 2, 2, and 22 for solvent control, 500, 1,600, 3,000, and 4,000 ug/ml (+S9, 15.0 hrs); Trial 3: percent cells with aberrations was 0, 1, 0, and 1 for solvent control, 250, 500, and 1,600 ug/ml (-S9, 12.0 hrs) [highest concentration was cytotoxic] and 0, 0, 2, and 52 for solvent control, 1,600, 3,000, and 4,000 ug/ml (+S9, 12.3 hrs) [highest concentration was cytotoxic]; Trial 4:percent cells with aberrations was 0, 2, 1, and 1 for solvent control, 500, 1,600, and 3,000 mg/ml (-S9, 17.0 hrs) [highest concentration was cytotoxic] and 3, 4, 3, and 32 for solvent control, 1,600, 3,000, and 4,000 ug/ml (+S9, 18.0 hrs) [highest concentration was cytotoxic].

Test condition: Metabolic activation: S9 from Aroclor 1254-induced male SD rat liver
Without S9, treated cells were incubated for 8-10 hours, then washed, and fresh medium was added containing colcemid for incubation of 2-3 hours. With S9, treated cells were incubated for 2 hours, then washed, fresh medium was added and incubation continued another 8-10 hours and colcemid was added for the last 2-3 hours. DMSO was the solvent control and both Mitomycin C and cyclophosphamide were used as positive controls. Total cells, number of aberrations, aberrations per cell and percent cells with aberrations were reported.

Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)

Conclusion: Benzyl alcohol induced chromosomal aberrations only at relatively high concentrations and the positive finding was only reproducible when metabolic activation was present.

Reliability: (1) valid without restriction
NTP study

08-JUL-2001 (29)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA98
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: negative

Method: other: Ames assay (modified) Not reported
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: No mutagenicity was reported.
Test condition: Metabolic activation: liver microsomal enzymes from KC-500

treated rats and "certain co-factors"
The slight modification of the Ames assay was a preincubation of the samples for 15 min at 37 C prior to plating. Benzaldehyde was tested with and without metabolic activation.
Conclusion: Benzaldehyde was non-mutagenic in this assay.
Reliability: (4) not assignable
The data were published in a brief abstract with limited detail and the study was conducted prior to GLP and OECD guidelines.
03-JUL-2001 (39)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: No mutagenicity was reported.
Test condition: Metabolic activation: none
The test was not conducted in duplicate and was part of a larger study examining the mutagenicity of aqueous chlorination of organic compounds.
Conclusion: Benzaldehyde was non-mutagenic in this assay.
Reliability: (3) invalid
Assay was not conducted in accordance with current standards (lack of duplicates) and was not well described.
03-JUL-2001 (37)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA100, TA1537, TA1538, and TA98
Concentration: not reported
Metabolic activation: with
Result: negative

Method: other: Ames assay
Year: 1976
GLP: no
Test substance: other TS

Result: No mutagenic activity was reported.
Test condition: Benzyl alcohol was applied as 5 ul of liquid to the center of agar plates seeded with tester strains.
Metabolic activation: not reported
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was non-mutagenic in this assay.
Reliability: (2) valid with restrictions

Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the results were published in a peer-reviewed journal. Therefore the data are considered reliable.

03-JUL-2001 (27)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 10 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: other TS

Result: Benzyl alcohol produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-JUL-2001 (20)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 1.0 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: other TS

Result: Benzyl alcohol did not induce chromosomal aberrations.
Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized,

suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none

Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was not clastogenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-JUL-2001 (20)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: maximum concentration = 20 ul/disk
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1986
GLP: no
Test substance: other TS

Result: Benzyl alcohol was reported to have a inhibition zone difference of 5 mm and was considered to be weakly positive.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was reported to have weak DNA damaging potential.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.

04-JUL-2001 (56)

Type: other: mutation test
System of testing: bacterial E. coli WP2 uvrA (trp-)
Concentration: 1.0-8.0 mg/plate
Metabolic activation: no data
Result: positive

Year: 1986
GLP: no
Test substance: other TS

Result: Benzyl alcohol was considered negative since the ratio was 0.8.
Test condition: The results were considered positive if the ratio of maximal revertants to spontaneous revertants was <2.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was reported to have weak DNA damaging potential.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.
04-JUL-2001 (57)

Type: other: antimutation test
System of testing: bacterial E. coli WP2 uvrA (trp-)
Concentration: 0.5-2.5 mg/ml
Metabolic activation: with
Result: positive

Year: 1986
GLP: no
Test substance: other TS

Result: Benzyl alcohol was considered positive since the ratio was 20%.
Test condition: Metabolic activation: induction with AF-2
The results were considered positive if the ratio of minimal revertants to AF-2-induced revertants was <50%.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was reported to have antimutagenic effects in this assay.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.
04-JUL-2001 (57)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 21 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: other TS

Result: Benzyl alcohol produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Test substance: Benzaldehyde (data on metabolic precursor, benzyl alcohol)
Conclusion: Benzyl alcohol was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.
04-JUL-2001 (33)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 21 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzaldehyde produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Conclusion: Benzaldehyde was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.
04-JUL-2001 (34)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (arg-, trp-, recE-) and H17 (arg-, trp-, recE+)
Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1989
GLP: no
Test substance: other TS

Remark: The reviewer does not agree with the authors' conclusion of benzaldehyde's DNA damaging potential for several reasons: (1) the S-probit value (0.258) on which the conclusion was based just barely fell into the value range (0.200-0.592) listed in the criteria as having DNA damaging potential. Chemicals with strong DNA damaging potential had S-probit values of more than 0.593. (2) The value for "repaired survival" indicated no DNA damaging potential. (3) Chemicals tested by the authors as positive controls produced calculated values several fold higher than any calculated for benzaldehyde.

Result: Without metabolic activation, benzaldehyde did not show any DNA damaging potential; however, when metabolically activated, benzaldehyde was considered to show DNA damaging potential. The authors conclusion was based only on the evaluation of the S-probit which was at the low end of the range of values considered to have DNA damaging potential. The repaired survival value calculated for benzaldehyde was well below the range considered to have DNA damaging potential.

Test condition: Benzaldehyde was tested with and without metabolic activation. The data were evaluated several ways: (1) calculation of the 50% survival concentration from the benzaldehyde concentration giving 50% survival turbidity of Rec+ over benzaldehyde concentration giving 50% survival turbidity of Rec-; (2) probit analysis of the area enclosed (S-probit) between the plotted survival lines of Rec+ and Rec-; (3) mathematical calculation of "repaired survival" from a plot of mean lethal hits of Rec- against survival as the difference between Rec- and Rec+ curves; and (4) quantitative evaluation of DNA damaging potential expressed as Rec-gram=S-probit/benzaldehyde concentration giving 50% survival turbitity of Rec-.
Metabolic activation: S9 mixture (not described)

Test substance: Benzaldehyde (JIS special grade)

Conclusion: Benzaldehyde was reported to show DNA damaging potential based on one parameter examined. The weight of evidence indicates that benzaldehyde does not have DNA damaging potential.

Reliability: (4) not assignable
Although the study was well documented, the methodolgy used was not standard and the results were open to interpretation.

5.6 Genetic Toxicity 'in Vivo'

Type: other: Clastogenic assay
Species: other: Mouse/ddY **Sex:** male
Route of admin.: unspecified
Exposure period: 24 hours (single dose); "appropriate time" (multiple dose)
Doses: Single dose: 0, 50, 100 or 200 mg/kg bw; 4 repeated doses: 100 mg/kg bw
Result: negative

Method: other: Micronucleus test
Year: 1988
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. MNPCE frequency was compared with the binomial distribution of historical control data (considered positive if $p<0.01$) and the dose-response relationship was tested by the Cochran-Armitage trend test (considered positive if $p<0.05$).
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: Single dose: MNPCE was 0.23, 0.23, 0.27, and 0.12% and PCE was 48.8, 55.5, 51.8, and 48.7% for 0, 50, 100, and 200 mg/kg bw; Multiple dose: MNPCE was 0.2% and PCE was 63.1% for 100 mg/kg bw
Test condition: Benzyl alcohol was administered by intraperitoneal injection either as a single dose or by 4 injections with 24-hour intervals between administration to 6 8-week-old male mice per treatment group. At harvest, bone marrow cells from the femur were taken and put onto glass slides where they were fixed in methanol (5 min) and stained with Giemsa. Slides were evaluated blind under light microscope (X100) for the number of micronucleated polychromatic erythrocytes (MNPCEs) and the proportion of polychromatic erythrocytes (PCEs). PCEs were evaluated in 1000 erythrocytes. MNPCE frequency of negative and positive controls was compared to historical controls in order to validate the method.
Test substance: Benzaldehyde (data for structurally related substance benzyl alcohol)
Conclusion: When administered by ip injection to mice either as a single dose or repeat dose, benzyl alcohol did not increase MNPCE frequency.
Reliability: (2) valid with restrictions
The data were well documented and the methodology used followed standard protocols. In addition, the results were published in a peer-reviewed journal.

09-JUL-2001 (17)

Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** male
Route of admin.: oral feed
Exposure period: 3 days
Doses: 1,150 ppm
Result: negative

Method: other: Sex-linked recessive lethal (SLRL) assay
Year: 1985
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Cluster analysis by Poisson distribution; normal test of lethal frequencies after clusters removed.
Test condition: Glass fiber discs, saturated with the test compound carried a 5% sucrose solution were used to expose day-old males (Canton-S) for 3 days in glass shell vials. Males were mated immediately after treatment with 3 new females for each of 3 broods. If no wild-type males were identified among 20 or more Basc males or Basc1 +/- females, then it was considered a lethal mutation. If a few F2 or F3 wild-type males survived at <5% of Basc males or Basc1 +/- females then it was considered a lethal mutation.
Test substance: Benzaldehyde (72-91% purity)
Conclusion: No induction of SLRL in Drosophila melanogaster.
Reliability: (1) valid without restriction
Part of NTP testing program.
30-MAR-2001 (54)

Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** male
Route of admin.: unspecified
Exposure period: Single dose
Doses: 2,500 ppm
Result: negative

Method: other: Sex-linked recessive lethal (SLRL) assay
Year: 1985
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Appropriate statistical evaluations: Yes. Cluster analysis by Poisson distribution; normal test of lethal frequencies after clusters removed.
Test condition: Males (Canton-S) aged 2-3 days were intraperitoneally injected with a 0.7% NaCl solution containing benzaldehyde. At 24-48 hours post injection males were mated with 3 new females for each of 3 broods. If no wild-type males were identified among 20 or more Basc males or Basc1 +/- females, then it was considered a lethal mutation. If a few F2 or F3 wild-type males survived at <5% of Basc males or Basc1 +/- females then it was considered a lethal mutation.
Conclusion: No induction of SLRL in Drosophila melanogaster.

Reliability: (1) valid without restriction
Part of NTP testing program.
30-MAR-2001 (54)

Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** male
Route of admin.: oral feed
Exposure period: 48 to 72 hours
Doses: 5,000 ppm (feed); 8,000 ppm (injection)
Result: ambiguous

Method: other: Sex-linked recessive lethal (SLRL) assay
Year: 1994
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Poisson analysis and binomial distribution.
Remark: No induction of recessive lethals with benzyl alcohol through feeding or injection.
Test condition: Benzyl alcohol was applied to 2 or 3 glass fibre filter disks in a 5% sucrose solution in glass vial. Solutions were renewed at 24 and 48 hours and males (Canton-S) were exposed for 72 hours after which males were mated with 3 virgin Basc females and transferred to fresh females every 2-3 days for a total production of 3 broods. No more than 100 F1 females were mated over the 3 broods from any P1 male in order to avoid recovery of multiple lethals from 1 male. If the number of wild-type males was 0, 1, or <5% of the Basc males, then the F2 cultures were scored as presumptive lethals. If feed exposure was non mutagenic, males were injected with benzyl acetate in a 0.7% NaCl solution. After 24 hours, surviving males were mated. Controls received solutions without benzyl alcohol.
Test substance: Benzaldehyde (data for structurally related substance benzyl alcohol, purity 99.8%)
Conclusion: Benzyl alcohol was non mutagenic in the SLRL assay.
Reliability: (1) valid without restriction
NTP study
09-JUL-2001 (10)

5.8.2 Developmental Toxicity/Teratogenicity

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5.8.3 Toxicity to Reproduction, Other Studies

Type: other: 1-generation
In Vitro/in vivo: In vivo
Species: rat
Strain: other: white **Sex:** male
Route of administration: gavage
Exposure period: Premating exposure, Females: Not described Premating exposure, Males: Not described
Frequency of treatment: Every other day
Duration of test: 32 weeks
Doses: 2 mg/rat, every other day (approximately 5 mg/kg bw/d) Actual dose: Approximately 5 mg/kg bw/d
Control Group: other: Ten control animals received only the vehicle oil.

Method: other: Reproductive toxicity
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
There was no statistical significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant; however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the parameters measured.
Result: Offspring toxicity F1 and F2: No effects reported.
Parental data and F1: Fewer treated females became pregnant; however, significance could not be determined.
Test condition: Two mg benzaldehyde was administered by gavage to 10 breeding age rats every other day (approximately 5 mg/kg bw/d) for a period of 32 weeks. Ten control animals received only the vehicle oil. Two pregnancies per rat were studied, one at 75 days and one at 180 days. The parameters examined included the number of pregnant females, number of offspring born, pup body weights at days 7 and 21 postpartum, and pup vitality.
Conclusion: It was concluded that treatment did not affect reproduction.
Reliability: (4) not assignable
Study translated from foreign article with limited descriptions.

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IUCLID Data Set

Existing Chemical ID: 123-11-5
CAS No. 123-11-5
EINECS Name anisaldehyde
EC No. 204-602-6
Molecular Formula C8H8O2

Producer Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Substance Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Memo: Benzyl Derivatives

Printing date: 16-NOV-2001
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Chapter (profile): Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 0 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

2.2 Boiling Point

Value: = 248 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

2.4 Vapour Pressure

Value: = .041 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated/Mean of Antoine & Grain method
Test condition: Calculated based on a measured boiling point of 248 C.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (23)

Value: = .044 hPa at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (14)

2.5 Partition Coefficient

log Pow: = 1.76 at 25 degree C

Method: other (measured)
Year: 1995
GLP: no data

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (6)

log Pow: = 1.79 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (22)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 4290 mg/l at 25 degree C

Method: other
Year: 1992
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (27)

Solubility in: Water
Value: = 2728 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a log Kow = 1.76
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (24)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 5.2 hour(s)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (19)

3.1.2 Stability in Water

Type: abiotic

Method: other:
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Substance is an aldehyde and will not hydrolyze in water.
16-NOV-2001

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1370000
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10% Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.26
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10% Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 7.08
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10%
Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.000056
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10%
Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.88
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10%
Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.13
Aerosol =0.000071% Air =2.6% Fish =0.00027% Sediment =0.10%
Soil =4.72% Suspended Sediment =0.0033% Water =92.6%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (18)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year: 1994
GLP: no data
Test substance: other TS

Result: Degradation % after time: 94.9% at 28 days
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Innoculum: 10% by volume of secondary effluent from an unacclimatised activated sludge
The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 C. The mean percentage biodegradation was calculated from 4 vessels on day 28.
Test substance: p-Methoxybenzaldehyde (99% purity)
Conclusion: p-Methoxybenzaldehyde is classified as readily and ultimately biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines and is considered reliable.
16-NOV-2001 (15)

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 1.1; nonlinear - 1.0. Expert survey results: ultimate - 2.9 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: p-Methoxybenzaldehyde is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (20)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 96 hour LC50 = 14.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (21)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 48 hour LC50 = 10.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (21)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = 110.07 -

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: p-Methyoxybenzaldehyde
Conclusion: 96 hour EC50 = 110.07 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (21)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

Type: LD50
Species: rat
Strain: Osborne-Mendel
Sex: male/female
No. of Animals: 5
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by the Litchfield and Wilcoxon (1949) method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Slope function: 1.2 (95% C.L. 1.1-1.3). Toxic signs were depression. Time of deaths was between 4 hours and 18 hours.
Result: LD50 = 1510 mg/kg bw (95% C.L. 1360-1700)
Number of deaths at each dose level: Not reported
Test condition: Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
30-JUN-2001 (10)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: male/female
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by the Litchfield and Wilcoxon (1949) method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Slope function: 1.6 (95% C.L. 1.2-2.0). Depression was observed within 2 hours Time of deaths was between 1 and 3 days.
Result: LD50 = 1260 mg/kg bw (95% C.L. 937-1700)
Number of deaths at each dose level: Not reported
Test condition: Groups of guinea pigs consisting of both males and females were fasted for 18 hours prior to treatment. Animals were

observed for toxic signs and death. The observation period was up to 2 weeks.
Not reported

Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (9)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 4
Vehicle: other:None

Method: other: LD50 calculated
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: No clinical signs were reported other than skin irritation which was graded as slight to moderate redness and moderate edema.
Result: LD50 = >5000 mg/kg bw
Number of deaths at each dose level: 1/4 deaths at 5000 mg/kg bw
Test condition: Four rabbits were topically administered 5 g/kg bw of test substance.
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results.Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.

30-JUN-2001 (12)

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 27-28 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 1,000 ppm Actual dose: approximately 50 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0, or 1,000 ppm for 27-28 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Conclusion: No effect reported at 1,000 ppm anisaldehyde in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (5)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 15 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 10,000 ppm Actual dose: approximately 500 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0, or 10,000 ppm for 15 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Conclusion: No effect reported at 10,000 ppm anisaldehyde in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (5)

5.5 Genetic Toxicity 'in Vitro'

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: up to 100 uM
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Sister chromatid exchange Student's t-test
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: p-Methoxybenzaldehyde by itself did not induce sister chromatid exchange. When added to MMC-pretreated cells, a dose-dependent increase in the frequency of SCEs was observed. Anisaldehyde was most effective at low concentrations (10 uM).

Test condition: Anisaldehyde was dissolved in DMSO and cells were exposed for 22 hours. The cells were prepared for analysis of sister chromatid exchange using a modified Giemsa staining method (Sakanishi and Takayama, 1977). In addition, cells pre-treated with mitomycin C (MMC) were also exposed to anisaldehyde to test for antimutagenic effects
Metabolic activation: with and without rat liver microsome fraction S9
Conclusion: p-Methoxybenzaldehyde is not an inducer of sister chromatid exchanges; however, it appears to increase the frequency of sister chromatid exchanges in cells pretreated with MMC.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (17)

Type: Mouse lymphoma assay
System of testing: non bacterial Mouse lymphoma cells L5178Y
Concentration: 0, 4.01, 5.02, 6.02, 7.02, or 8.03 umol/L
Cytotoxic Concentration: 7.02 mmol/L
Metabolic activation: without
Result: negative

Method: other: Mouse lymphoma assay (Clive and Spector, 1975)
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: No effect at or below 6.02 mmol/L. Positive effects at 7.02 and 8.03 mmol/L.
Test condition: Metabolic activation: none
Test chemicals were dissolved in Fischer’s medium without serum, water, ethanol, or DMSO. The solvent for 4-methoxybenzaldehyde was not specified. Cells were treated with or without S9 and then exposed for 3 hours to 4-methoxybenzaldehyde. Viability was estimated by dying a sample of the cells with trypan blue to determine the number of cells that exclude the dye. Alkaline unwinding and hydroxyapatite elution were used to detect strand breaks. In order to determine the relative DNA content, radioactivity was measured using a liquid scintillation counter. DNA damaging potential was determined by using 4 criteria: 1) positive if the relative fraction of ssDNA increased by 6.5% and relative toxicity was <5%; 2) positive if at 2 or more concentrations, the increase in relative fraction of ssDNA is greater than the increase in relative toxicity when relative toxicities are 5 to 50% but equivocal if seen only at 1 concentration; 3) if the increase in relative fraction of ssDNA is dose-related, then (1) and (2) are correct; and 4) negative if cytotoxic and no i
Conclusion: p-Methoxybenzaldehyde produced an increase in DNA strand breaks at cytotoxic concentrations of 7.02 and 8.03 mmol/L.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
04-APR-2001 (4)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA97, TA102
Concentration: 0, 0.001, 0.005, 0.01, 0.5, or 1 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay Data were evaluated using the Kruskal-Wallis test and regression analysis.
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: p-Methoxybenzaldehyde produced no increase in mutagenesis, with or with out S9 mix.
Test condition: Cells preincubated with or without S9 mix were exposed to anisaldehyde dissolved in DMSO for 20 minutes.
Metabolic activation: S9 mix
Conclusion: p-Methoxybenzaldehyde was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Japanese article with limited translation and tabulated results.
04-APR-2001 (3)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA98, TA100
Concentration: 3 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Spot tests are less sensitive than quantitative experiments. Also, the results from strain TA100 are difficult to interpret because the high growth background (150-200 colonies per plate); however, spot tests provide a good "screening" method for large numbers of chemicals.
Result: p-Methoxybenzaldehyde produced a negative response in this assay.
Test condition: For each experiment viable count was determined, the number of spontaneous revertants was measured, the presence of the rfa-mutation was determined by crystal violet inhibition, the presence of the plasmid pKM 101 in strains TA98 and TA100 was determined by resistance to ampicillin, and the

response to positive controls N-methyl-N-nitrosoquanidin (without metabolic activation) and 2-aminoanthracene (with activation) was determined. Spectroscopic-grade ethanol was used as the solvent. The test substance was tested at 3 umol/plate in TA98, TA100, TA1535, and TA1537 with or without S9. If there was no background lawn of bacteria, the tests were redone using lower concentrations. Uncertain results prompted the conduction of the tests at 4 concentration levels (0.03, 0.3, 3 and 30 umol/plate). Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats

Conclusion: p-Methoxybenzaldehyde is non-mutagenic in the Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 with or without S9.

Reliability: (1) valid without restriction
Study is published in a peer reviewed journal with adequate description and follows standard procedures.

30-MAR-2001 (2)

Type: other: clastogenic assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 50 nM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Chromsomal aberrations Chi square test
Year: 1982
GLP: no
Test substance: other TS

Result: p-Methoxybenzaldehyde produced a signficant difference in chromosomal abberations as compared to the vehicle control ($p<0.001$).

Test condition: DMSO was used as the solvent and control. Cells were exposed to the flavoring agent for 24 hours and then incubated another 24 hours without the flavor after which the cells were treated with colchicine for 2-3 hours. Cells were staining using Giemsa staining method. The scoring of about 200 metaphase spreads, containing 20-26 chromosomes were used to calculate the percentage of chromosomal aberrations.
Metabolic activation: rat liver microsome fraction S9

Test substance: p-Methoxybenzaldehyde (90-95% purity)

Conclusion: p-Methoxybenzaldehyde induced chromosomal aberrations.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.

02-APR-2001 (11)

Type: Mouse lymphoma assay
System of testing: non bacterial Mouse lymphoma cells L5178Y
Concentration: 0, 1.010E-3, 3.070E-3, 3.560E-3, 4.040E-3, 4.560E-3, or 5.080E-3 mol/L
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Mouse lymphoma assay (Clive et al., 1979) The data were evaluated by testing for normal distribution (Shapiro and Wilk, 1965) and subjected to analysis of variance. A pairwise two-tailed Student's t-test was also performed. Linear,
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: At concentrations of 3.560E-3 mol/L and higher, p-methoxybenzaldehye significantly increased the mutation frequency by 3- to 4-fold.
Test condition: Metabolic activation: none
The method was slightly modified by resuspending the cells in Fischer's medium (10% horse serum) after a 4-hour exposure to 4-methoxybenzaldehyde. Hepes was added to the suspension and the concentration of NaHCO3 was reduced. Growth rate of the cells was improved by altering the pH of the culture medium from 6.8 to 7.2. During the expression period of 48 hours, the cell concentrations were adjusted daily. Trifluorothymidine was added as the selective agent prior to preparing the final plates. Plates with trifluorothymidine were counted manually and those without were counted with an automatic colony counter. Total survival and mutation frequency were calculated.
Conclusion: Based on the results of this study, the authors concluded that a 2- to 4-fold increase in mutation frequency in this assay is" less predictive of carcinogenicity unless the compound requires metabolic activation to be mutagenic or gives results that are difficult to reproduce".
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

04-APR-2001 (25)

Type: Sister chromatid exchange assay
System of testing: human lymphocytes Human lymphocytes
Concentration: 0-2.0 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange (Jansson et al., 1986) The data were analyzed using linear regression by least squares and significance was tested at $p<0.05$, 0.01, and 0.001.
Year: 1988
GLP: no
Test substance: other TS

Result: Statisticially signficant increase in sister chromatid exchanges ($p<0.001$) as compared to the vehicle control. The regression coefficient was 4.5 SCE/cell/mM.
Test condition: DMSO and ethanol were used as solvents and negative controls. The positive control used was styrene-7,8-oxide. After an exposure of 88 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified), 25 metaphases from one culture were analysed.
Metabolic activation: Phytohemagglutinin-stimulated
Test substance: p-Methoxybenzaldehyde (96% purity)
Conclusion: p-Methoxybenzaldehyde induced sister chromatid exchanges in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
01-APR-2001 (8)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 5 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: p-Methoxybenzaldehyde produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the

plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.

Conclusion: p-Methoxybenzaldehyde was non mutagenic.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-APR-2001 (7)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 0.5 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: p-Methoxybenzaldehyde did not induce chromosomal aberrations.

Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none

Conclusion: p-Methoxybenzaldehyde was not clastogenic in this assay.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-APR-2001 (7)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 0.05-500 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1982
GLP: no
Test substance: other TS

Result: p-Methoxybenzaldehyde did not increase the incidence of mutation as compared to the vehicle controls, either with or without S9 mix.
Test condition: DMSO was used as the solvent and control. The results were considered positive if a reproducible, dose-related increase in the number of revertants and a greater than 2-fold increase in spontaneous mutation rate was observed. Metabolic activation: rat liver microsome fraction S9 from Aroclor induced rats
Test substance: p-Methoxybenzaldehyde (90-95% purity)
Conclusion: p-Methoxybenzaldehyde was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.
02-APR-2001 (11)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: No mutagenicity was reported.
Test condition: Metabolic activation: none
The test was not conducted in duplicate and was part of a larger study examining the mutagenicity of aqueous chlorination of organic compounds.
Conclusion: p-Methoxybenzaldehyde was non-mutagenic in this assay.
Reliability: (3) invalid
Assay was not conducted in accordance with current standards (lack of duplicates) and was not well described.
03-JUL-2001 (16)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 22 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: p-Methoxybenzaldehyde produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Conclusion: p-Methoxybenzaldehyde was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.
04-JUL-2001 (13)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** no data
Route of admin.: oral feed
Exposure period: Not reported
Doses: 5 mM
Result: negative

Method: other: Sex linked recessive lethal mutation assay (Wuergler et al., 1977)
Year: 1983
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Remark: p-Ethoxybenzaldehyde did not increase the number of sex-linked recessive lethal mutations as compared to controls.
Test condition: Flies were exposed to the test compound prepared in a 5% saccharose solution and 2% ethanol and 2% Tween 80 for compounds with poor water solubility. Further details of the methodology were not reported.
Test substance: p-Methoxybenzaldehyde (data for structurally related substance p-ethoxybenzaldehyde)
Conclusion: p-Ethoxybenzaldehyde did not induce sex linked recessive

lethals in Drosophila melanogaster.

Reliability: (2) valid with restrictions
The data were acquired by standard methodology and published in a peer reviewed journal but there was a limited description of the protocol and the results were tabulated.

30-MAR-2001 (26)

5.8.2 Developmental Toxicity/Teratogenicity

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5.8.3 Toxicity to Reproduction, Other Studies

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IUCLID Data Set

Existing Chemical ID: 121-33-5
CAS No. 121-33-5
EINECS Name vanillin
EC No. 204-465-2
Molecular Formula C8H8O3

Producer Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Substance Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Memo: Benzyl Derivatives

Printing date: 16-NOV-2001
Revision date:
Date of last Update: **16-NOV-2001**

Chapter (profile): Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 80 - 81 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (27)

Value: = 81.5 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (2)

2.2 Boiling Point

Value: = 285 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (2)

2.4 Vapour Pressure

Value: = .0002 hPa at 25 degree C

Method: other (measured)
Year: 1994
GLP: no data
Test substance: other TS

Method: Measured
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (37)

Value: = .0006 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a measured boiling point of 285 C.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (33)

2.5 Partitio n Coefficient

log Pow: = 1.05 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (32)

log Pow: = 1.21 at 25 degree C

Method: other (measured)
Year: 1995
GLP: no data

Method: Measured
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (6)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 11000 mg/l at 25 degree C

Method: other
Year: 1992
GLP: no data
Test substance: other TS

Method: Measured
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (36)

Solubility in: Water
Value: = 6875 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a log Kow = 1.21
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (34)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 4.7 hour(s)

GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (29)

3.1.2 Stability in Water

Type: abiotic

Method: other:
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Substance is an aldehyde and will not hydrolyze in water.
16-NOV-2001

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.04E+08
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.64
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.0
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 8.93E-08
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.81
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.32
Aerosol =9.11E-06% Air =0.0044% Fish =0.000080% Sediment =0.031% Soil =1.42% Suspended Sediment =0.00098% Water =98.5%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (28)

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 1.2; nonlinear - 1.0. Expert survey results: ultimate - 2.9 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (30)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Static system with fathead minnows
Species: Pimephales promelas (Fish, fresh water)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1976
GLP: no
Test substance: other TS

Result: No experimental data specific to test substance cited in EPA report.
Test condition: Fish tested and acclimated in reconstituted water (NaHCO3, CaSO4 added to distilled water).
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: The 96 hour LC50 was reported to be 116 mg/L.
Reliability: (2) valid with restrictions
EPA study.
16-NOV-2001 (20)

Type: other: Static system with fathead minnows
Species: Pimephales promelas (Fish, fresh water)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1976
GLP: no
Test substance: other TS

Result: No experimental data specific to test substance cited in EPA report.
Test condition: Fish tested and acclimated in in Lake Superior water. A low DO was noted.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: The 96 hour LC50 was reported to be 112 mg/L.
Reliability: (2) valid with restrictions
EPA study.
16-NOV-2001 (19)

Type: other: Static system with fathead minnows
Species: Pimephales promelas (Fish, fresh water)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1976
GLP: no
Test substance: other TS

Result: No experimental data specific to test substance cited in EPA report.
Test condition: Fish tested in reconstituted water (NaHCO3, CaSO4 added to distilled water) but acclimated in Lake Superior water. A low DO was noted.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: The 96 hour LC50 was reported to be 88 mg/L.
Reliability: (2) valid with restrictions
EPA study.
16-NOV-2001 (20)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Result: 96 hour LC50 = 23.0
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (31)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: 48 hour LC50 = 17.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (31)

Type: other: EC50 for prevention of attachment of zebra mussel
Species: other: Zebra mussel (Dreissena polymorpha)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1997
GLP: no
Test substance: other TS

Remark: Biological observations: No effect
Test condition: Fifteen zebra mussels with 5-8 mm shell length were exposed at 17 C for 48 hours to test chemical followed by a 48 hour post exposure period in untreated well water (pH = 7.9; alkalinity as CaCO3 = 107 mg/L; hardness as CaCO3 =134 mg/L; conductivity = 281 uS/cm). Two replicates were conducted. Inhibition of attachment was assess by touching mussels with a blunt probe.
Nominal concentrations as mg/L: 0-50 (specific values not reported)
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Vanillin did not prevent the attachment of zebra mussels.
Reliability: (2) valid with restrictions
Published data and reasonably well described.
27-APR-2001 (1)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = 378 -

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: 96 hour EC50 = 378 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (31)

Species: other aquatic plant: Chlorella vulgaris
Unit: **Analytical monitoring:** no data

Method: other: Growth and cell division
Year: 1971
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: None reported
Control response: Unknown
Remark: Biological observations: Growth inhibition of 0, 0 and 50% after 80 hours and 0, 0 and 30% after 160 hours at 0.00005, 0.0001, and 0.001 M, respectively.
Endpoint basis: Inhibition of cell growth
Test condition: Cell growth was determined by direct cell counting using a Burker counting chamber.
Exposure period: 80 and 160 hours
Nominal concentration: 0.00005, 0.0001, and 0.001 M
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Vanillin inhibited growth of Chlorella vulgaris at the highest concentration tested.
Reliability: (2) valid with restrictions
Published data and reasonably well described. No statistics performed.
14-MAR-2001 (3)

Species: other aquatic plant: Chlorella vulgaris
Exposure period: 5 hour(s)
Unit: **Analytical monitoring:** yes

Method: other: Respiration of photosynthesizing cells
Year: 1971
GLP: no
Test substance: other TS

Method: Analytical monitoring: Warburg respirometer
Appropriate statistical evaluations: None reported
Control response: Unknown
Remark: Biological observations: Stimulation of respiration of 0, 5, and 100% at pH 5.6 and 0, 5, and 165% at pH 7.2 at 0.00005, 0.0001, and 0.001 M, respectively.
Endpoint basis: Stimulation of cell respiration (oxygen uptake)
Test condition: Cells were maintained in the dark at 25 C in 20 ml flasks and oxygen uptake was measured over a period of 5 hours at a pH of 5.6 or pH of 7.2.
Nominal concentration: 0.00005, 0.0001, and 0.001 M
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Vanillin strongly enhances respiration at the highest dose tested and appears to be pH-dependent.
Reliability: (2) valid with restrictions
Published data and reasonably well described. No statistics performed.
14-MAR-2001 (3)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: mouse
Strain: other: BDF1
Sex: male
Vehicle: other:2% Tween 80

Method: other: LD50 calculated by using the Lorke method
Year: 1988
GLP: no
Test substance: other TS

Result: LD50 = approximately 1000 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: Not reported
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (2) valid with restrictions
Although the details of the LD50 determination were not described, the LD50 was determined as part of another study conducted to examine MMC induction. The induction study appears to follow current standards and therefore the results of the LD50 determination are considered reliable.
30-JUN-2001 (10)

Type: LD50
Species: rat
Strain: Osborne-Mendel
Sex: male/female
No. of Animals: 5
Vehicle: other:Propylene glycol (20%)
Route of admin.: other: Gavage

Method: LD50 calculated by the Litchfield and Wilcoxon method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: other TS

Remark: Slope function: 1.3 (95% C.L. 1.2-1.5). Coma was reported soon after treatment. Time of deaths was between 4 hours and 4 days.

Result: LD50 = 1580 mg/kg bw (95% C.L. 1390-1810)
Number of deaths at each dose level: Not reported
Test condition: Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment Animals were observed for toxic signs and death. The observation period was 2 weeks.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (16)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: male/female
Vehicle: other:Propylene glycol (20%)
Route of admin.: other: Gavage

Method: LD50 calculated by the Litchfield and Wilcoxon method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: other TS

Remark: Slope function: 1.1 (95% C.L. 1.09-1.2). Depression was reported within 1 hour. Time of deaths was between 1 and 3 days.
Result: LD50 = 1400 mg/kg bw (95% C.L. 1310-1500)
Number of deaths at each dose level: Not reported
Test condition: Groups of guinea pigs consisting of both males and females were fasted for 18 hours prior to treatment Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Not reported
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (16)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

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5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 27-28 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 1,000 ppm Actual dose: approximately 50 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 1,000 ppm for 27-28 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: No effect reported at 1,000 ppm vanillin in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (5)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 16 weeks
Frequency of treatment: daily
Post exposure period: None
Doses: 10,000 ppm Actual dose: approximately 500 mg/kg bw/d
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male and 5 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0 or 10,000 ppm for 16 weeks. No vehicle was used. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and hematocrits) were performed at the termination of the study. Macroscopic examination of all tissues was performed. Histopathological
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: No effect reported at 10,000 ppm vanillin in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (5)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 1 year
Frequency of treatment: daily
Post exposure period: None
Doses: 20,000 or 50,000 ppm
Control Group: other: basal diet

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 5 male Osborne-Mendel rats were provided test substance in the diet at concentrations of 0, 20,000 or 50,000 ppm for 1 yr. Corn oil (3%) was added to control and test diet as a binder to reduce evaporation of the flavoring. The diet was
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: No effect reported at up to 50,000 ppm vanillin in the diet of rats.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (5)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily
Post exposure period: None
Doses: 5,000, 10,000, or 20,000 ppm
Control Group: other: diet only

Method: other: Screening method used by U.S. Food and Drug Administration.
Year: 1967
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects were reported.
Test condition: Groups of 12 male and 12 female Osborne-Mendel rats were provided test substance in the diet at concentrations of 0,

5,000, 10,000, or 20,000 ppm for 2 yrs. No vehicle was used. Propylene glycol (3%) was added to control and test diet as a binder to reduce evaporation of the flavoring. The diet was prepared and analyzed weekly. Measurements of body weight, food intake and general condition were recorded weekly. Hematological examinations (white cell counts, red cell counts, hemoglobins and

Test substance: m-Methoxy-p-hydroxybenzaldehyde

Conclusion: No effect reported at up to 20,000 ppm vanillin in the diet of rats.

Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (5)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: albino/Carworth Farm)
Route of administration: oral feed
Exposure period: 26 weeks
Frequency of treatment: ad libitum
Post exposure period: None
Doses: 0, 0.1, 0.5, or 1.0% in the diet (approximately 0, 40, 214, or 437 mg/kg bw/d) Actual dose: 0, 40, 214, or 437 mg/kg bw/d
Control Group: other: basal diet only

Method: other: 26-week feeding study
Year: 1955
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results were analyzed with Fisher Student "t".

Result: Toxic response/effects by dose level: Mild respiratory infections were reported throughout all test groups including controls. There were no differences (p=0.05) in survival, body weight, food consumption, and pathology between treated animals and controls.

Test condition: Groups of 10 male rats were fed diets containing 0, 0.1, 0.5, or 1.0% vanillin in the diet (approximately 0, 40, 214, or 437 mg/kg bw/d, respectively) for a period of 26 weeks. Clinical signs, body weight, and food consumption were recorded. Tissues from 5 randomly selected rats from each group were preserved in formalin and tissues (heart, liver, kidney, spleen, and large and small intestines) from 3 rats underwent histopathological examination.

Test substance: m-Methoxy-p-hydroxybenzaldehyde

Conclusion: Vanillin administered at up to 1.0% in the diet of rats over 26 weeks produced no evidence of toxicity.

Reliability: (2) valid with restrictions
The study was conducted prior to GLP guidelines and the results were presented in a summary; however, the data were

considered reliable.

28-MAR-2001 (7)

Type: Sub-acute
Species: rat **Sex:** male
Strain: no data
Route of administration: other: inhalation
Exposure period: 4 months
Frequency of treatment: not reported
Post exposure period: Not reported
Doses: 0.5, 1.5, or 15 mg/m3
Control Group: other: not reported

Method: other: 4-month inhalation study
Year: 1982
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: LOAEL: 1.5 mg/m3
NOAEL: 0.5 mg/m3
Toxic response/effects by dose level: At highest dose, animals showed effects on the nervous and cardiovascular systems and effects on liver, adrenal gland, and hematopoetic function (not described).
Test condition: Groups of rats were exposed to 0.5, 1.5, or 15 mg/m3 in inhalation chambers for 4 months.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: The author reported that an inhalation concentration of 1.5 mg/m3 in rats approaches the toxicological threshold for vanillin whereas 0.5 mg/m3 showed no effects.
Reliability: (4) not assignable
The results were reported from a Russian article in a very short English abstract.

11-MAR-2001 (21)

Type: Sub-acute
Species: other: Dpg/Mongrel **Sex:** male
Strain: other: Mongrel
Route of administration: other: oral capsule
Exposure period: 26 weeks and 4 days
Frequency of treatment: daily, 5 days per week
Post exposure period: None
Doses: 0, 25, or 100 mg/kg bw/d
Control Group: other: 0 mg/kg bw/d

Method: other: 26-week oral toxicity study
Year: 1955
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No effects due to treatment were reported.
Test condition: Groups of 1 male and 1 female dog were administered 0, 25, or 100 mg vanillin/kg bw/d by capsule, 5 days/week for 26 weeks and 4 days. Dogs were observed for clinical signs and body weight was recorded. At study initiation, 13 weeks and study termination, complete blood counts, hematocrits, sedimentation rates, BUN, bromsulphalein liver function tests, icterus indexes, phenolsulfonphthalein kidney function tests, and urinalyses were performed. At necropsy, the liver, kidney, large and small intestine, heart and spleen were histologically examined.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Vanillin administered at up to 100 mg/kg bw/d to mongrel dogs for over 26 weeks produced no evidence of toxicity.
Reliability: (2) valid with restrictions
The study was conducted prior to GLP guidelines and the results were presented in a summary; however, the data were considered reliable.
28-MAR-2001 (7)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA98, TA100
Concentration: NG
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay Not reported
Year: 1985
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde did not increase the number of revertants.

Test condition: DMSO was used as the solvent. The positive control was benzo(a)pyrene. Plates were incubated for 48 hours after which revertant colonies were counted. Three replicates with 4 plates were done.
Metabolic activation: rat liver microsome fraction S9 from Aroclor induced rats
Test substance: m-Methoxy-p-hydroxybenzaldehyde (90% purity)
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
15-MAY-2001 (23)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Concentration: 10,000 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay Not reported
Year: 1989
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde was inactive in the Ames assay using Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100 with or without S9 acitivation.
Test condition: Following 2 days of incubation at 37 C, revertant colonies were counted electronically.
Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.
30-MAR-2001 (8)

Type: other: clastogenic assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 20 nM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Chromsomal aberrations Chi square test
Year: 1982
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde produced no signficant difference in chromosomal abberations as compared to the vehicle control
Test condition: DMSO was used as the solvent and control. Cells were exposed to the flavoring agent for 24 hours and then incubated another 24 hours without the flavor after which the cells were treated with colchicine for 2-3 hours. Cells were staining using Giemsa staining method. The scoring of about 200 metaphase spreads, containing 20-26 chromosomes was used to calculate the percentage of chromosomal aberrations.
Metabolic activation: rat liver microsome fraction S9
Test substance: m-Methoxy-p-hydroxybenzaldehyde (90-95% purity)
Conclusion: m-Methoxy-p-hydroxybenzaldehyde did not induce chromosomal aberrations.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.
02-APR-2001 (18)

Type: Sister chromatid exchange assay
System of testing: non bacterial Human lymphocytes
Concentration: 0-1.0 mM; confirmation experiment: 0.75 or 1.0 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange The results were analyzed using regression analysis and t-test with separate variance estimate.
Year: 1986
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde produced a dose-dependent induction of sister chromatid exchanges($p<0.01$; two-tailed test). In the confirmation experiment, vanillin was confirmed as a potent inducer of sister chromatid exchanges ($p,0.001$; two-tailed test). At 0.75 mM, vanillin produced 20 SCE per treated cell and at 1.0mM produced 20 SCE per 4 treated cells.
Test condition: DMSO, ethanol or DMSO/ethanol (1:1) were used as solvents

and negative controls. The solvent used with vanillin was not specified. The positive control used was styrene-7,8-oxide. After an exposure of 88-90 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified in first experiment), 25 metaphases from one culture were analysed. The first experiment used vanillin concentrations ranging from 0-1 mM. Since positive results were reported, a confirmation experiment was conducted using 2 concentrations of 0.75 and 1.0 mM.
Metabolic activation: Phytohemagglutinin-stimulated

Test substance: m-Methoxy-p-hydroxybenzaldehyde (99.6% purity)

Conclusion: m-Methoxy-p-hydroxybenzaldehyde was reported to be an inducer of sister-chromatid exchanges.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

30-MAR-2001 (14)

Type: Chromosomal aberration test
System of testing: non bacterial Human lymphocytes
Concentration: 0, 1, 2 or 4 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Chromosomal aberrations (Jansson et al., 1986) The data were analyzed using Fisher's exact probability test and the X2 test.
Year: 1987
GLP: no
Test substance: other TS

Remark: The significance of counting and including gaps is controversial and this parameter is not included in the evaluation of chromosome aberration tests according to the OECD guidelines for testing chemicals.

Result: In the first experiment and only at the highest concentration tested (4 mM), a statistically increase in chromosomal aberrations with gaps included ($p<0.01$) was reported. This effect was not repeated in the second experiment.

Test condition: Ethanol was used as the solvent and negative control. The positive control used was styrene-7,8-oxide. Following an exposure time of 48 hours, chromosome and chromatid aberrations and gaps were recorded. Two experiments, using 3 different concentrations, were conducted with the lymphocytes of two different donors. For each concentration, 100 cells were analyzed per slide
Metabolic activation: Phytohemagglutinin-stimulated

Test substance: m-Methoxy-p-hydroxybenzaldehyde

Conclusion: Although vanillin increased in chromosomal aberrations with gaps included at highest concentration in one experiment,

these findings were not repeated in a second experiment. Moreover, the inclusion of "gaps" in evaluating chromosomal aberrations is a controversial topic and has not been included in standard protocols for chromosomal aberration testing.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (13)

Type: Sister chromatid exchange assay
System of testing: non bacterial Human lymphocytes
Concentration: 0, 1 or 2 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange (Jansson et al., 1986)
Student's t-test
Year: 1987
GLP: no
Test substance: other TS

Result: There was a statistically signficant increase in sister chromatid exchanges at both 1 mM and 2 mM ($p<0.001$) as compared to the vehicle control. The number of sister chromatid exchanges per cell for control, 1 mM, and 2 mM was 14.3, 19.2, and 24.2, respectively. The positive control was 21.3 sister chromatid exchanges per cell.

Test condition: Ethanol was used as the solvent and negative control. The positive control used was styrene-7,8-oxide. After an exposure of 88 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified), 20 metaphases from one culture were analysed. Metabolic activation: Phytohemagglutinin-stimulated

Test substance: m-Methoxy-p-hydroxybenzaldehyde

Conclusion: m-Methoxy-p-hydroxybenzaldehyde at concentrations of 1 and 2 mM induced sister chromatid exchanges in human lymphocytes.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (13)

Type: Sister chromatid exchange assay
System of testing: human lymphocytes Human lymphocytes
Concentration: 0-1.0 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Sister chromatid exchange (Jansson et al., 1986) The data were analyzed using linear regression by least squares and significance was tested at $p<0.05$, 0.01, and 0.001.
Year: 1988
GLP: no
Test substance: other TS

Result: Statisticially signficant increase in sister chromatid exchanges ($p<0.01$) as compared to the vehicle control. The regression coefficient was 11.4 SCE/cell/mM.
Test condition: DMSO and ethanol were used as solvents and negative controls. The positive control used was styrene-7,8-oxide. After an exposure of 88 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified), 25 metaphases from one culture were analysed.
Metabolic activation: Phytohemagglutinin-stimulated
Test substance: m-Methoxy-p-hydroxybenzaldehyde (99% purity)
Conclusion: m-Methoxy-p-hydroxybenzaldehyde induced sister chromatid exchanges in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
01-APR-2001 (15)

Type: other: clastogenic assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 5, 20 or 40 nM
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: Chromsomal aberrations Chi square test in comparison with control values
Year: 1985
GLP: no
Test substance: other TS

Result: No statisically sigificant increase in chromosal aberrations ($p<0.05$ and $p<0.001$) was reported. At 5, 20, and 40 nM the numerical frequency of cells with chromosome aberrations was 4.2, 6.0 and 6.4, respectively.
Test condition: DMSO was used as the solvent and MNNG as the positive control. Cells were exposed for 24 hours. Prior to harvesting, the cells were treated with colchicine for 2 hours and fixated with ethanol/acetic acid. The cells were

stained using the Giemsa staining method and then karyotypes were analyzed by the high resolution banding method of Yunis (1981).
Metabolic activation: none

Test substance: m-Methoxy-p-hydroxybenzaldehyde (90-95% purity)
Conclusion: m-Methoxy-p-hydroxybenzaldehyde did not induce chromosomal aberrations.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (17)

Type: Unscheduled DNA synthesis
System of testing: non bacterial Rat hepatocyte (Fischer and Sprague-Dawley)
Concentration: 500 ug/ml
Cytotoxic Concentration: not reported
Metabolic activation: no data
Result: positive

Method: other: Unscheduled DNA synthesis (Williams, 1977, 1980 and Butterworth et al., 1987) Not reported
Year: 1989
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde treatment did not increase UDS compared to controls.
Test condition: Rat hepatocytes were incubated in culture dishes for 18-20 hours with vanillin. Concurrent cell counting or measurement of LDH release was used to determine relative cell survival. UDS was measured by electronically counting nuclear grains and calculating the net nuclear grain count (NNG). At each test concentration, 75-150 cells were analyzed. An increase in NNG of "at least 6 grains per nucleus above the concurrent solvent control value and/or an increase in the percent of nuclei having 6 or more net grains to at least 10% above the concurrent negative control" was considered a positive UDS response.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was not genotoxic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.

01-APR-2001 (9)

Type: Mouse lymphoma assay
System of testing: non bacterial L5178Y mouse lymphoma cell line
Concentration: 1000 ug/ml (without S9) and 1500 ug/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Mouse lymphoma assay (Clive et al. 1979) Not reported
Year: 1989
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde treatment did not affect mutagenic activity.
Test condition: Cells were exposed to vanillin for 4 hours, washed, incubated for 48 hours and then cloned. After 10-14 days, colonies were automatically counted. The ratio of mutant to viable colonies cloned without selective medium was considered to be the mutant frequency.
Metabolic activation: with and without rat liver microsome fraction S9 and cofactors
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology, but there was limited description of the study and the results were tabulated.
01-APR-2001 (9)

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: up to 100 uM
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Sister chromatid exchange Student's t-test
Year: 1987
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde by itself did not induce sister chromatid exchange. When added to MMC pretreated cells, a dose-dependent increase in the frequency of sister chromatid exchanges was observed. The highest increase (42%) occurred at a dose of 100 uM. There was no effect by vanillin on sister chromatid exchanges in cells pretreated by MMS or MNNG. An increased incidence of sister chromatid exchanges was observed in cells pretreated with EMS, ENNG, ENU and MNU suggesting that the sister chromatid exchange-enhancing effects of vanillin seemed to be dependent on the quality of the lesions of the DNA.
Test condition: Metabolic activation: with and without rat liver microsome

fraction S9
Vanillin was dissolved in DMSO and cells were exposed for 22 hours. The cells were prepared for analysis of sister chromatid exchange using a modified Giemsa staining method (Sakanishi and Takayama, 1977). In addition, cells pre-treated with mitomycin C (MMC) were also exposed to vanillin to test for antimutagenic effects. Further investigations on antimutagenicity were conducted by exposing cells pretreated with ethyl methanesulphonate (EMS), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), N-ethyl-N-nitrosourea (ENU), methyl methanesulphonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), or N-methyl-N-nitrosourea (MNU) to vanillin.

Test substance: m-Methoxy-p-hydroxybenzaldehyde

Conclusion: m-Methoxy-p-hydroxybenzaldehyde is not an inducer of sister chromatid exchanges; however, it appears to increase the frequency of sister chromatid exchanges in cells pretreated with MMC, EMS, ENNG, ENU and MNU, but not MMS or MNNG.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (26)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 0.05-1000 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1982
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde did not increase the incidence of mutation as compared to the vehicle controls, either with or without S9 mix.

Test condition: DMSO was used as the solvent and control. The results were considered positive if a reproducible, dose-related increase in the number of revertants and a greater than 2-fold increase in spontaneous mutation rate was observed. Metabolic activation: rat liver microsome fraction S9 from Aroclor induced rats

Test substance: m-Methoxy-p-hydroxybenzaldehyde (90-95% purity)

Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non mutagenic.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.

02-APR-2001 (18)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 0, 100, 333, 1000, 3333, or 10000 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1986
GLP: yes
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde produced no increased incidence of mutation as compared to the vehicle controls, either with or without S9 mix.
Test condition: Metabolic activation: rat liver microsome fraction S9 Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98), and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains without S9. 2-Aminoanthracene was used with all strains incubated with S9. Solvent controls were also prepared concurrently. Preliminary tests were conducted to assess the cytotoxicity of the test compound and establish suitable concentrations for testing. At least 5 concentrations of the test chemicals (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 C for 48 hours. A test chemical was considered “mutagenic” if there was a dose-related, reproducible increase in the number of revertants over background (not required to be 2-fold increase), “non mutagenic” if there was no increase, and “questionable” if there was no clear reproducible dose-related increase or “when the response was of insufficient magnitude to support a determination of mutagenicity”.
Test substance: m-Methoxy-p-hydroxybenzaldehyde (99% purity)
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non-mutagenic.
Reliability: (1) valid without restriction
NTP study
04-APR-2001 (22)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 10 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-APR-2001 (12)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 1.0 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde did not induce chromosomal aberrations.
Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was not clastogenic in this assay.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-APR-2001 (12)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: other TS

Result: No mutagenicity was reported.
Test condition: Metabolic activation: none
The test was not conducted in duplicate and was part of a larger study examining the mutagenicity of aqueous chlorination of organic compounds.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non-mutagenic in this assay.
Reliability: (3) invalid
The assay was not conducted in accordance with current standards (lack of duplicates) and was not well described.
03-JUL-2001 (25)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 21 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: other TS

Result: m-Methoxy-p-hydroxybenzaldehyde produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Test substance: m-Methoxy-p-hydroxy benzaldehyde
Conclusion: m-Methoxy-p-hydroxybenzaldehyde was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.
04-JUL-2001 (24)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Clastogenic assay
Species: other: Mice/BDF1 **Sex:** male
Route of admin.: unspecified
Exposure period: Single dose
Doses: Not reported
Result: ambiguous

Method: other: Chromosomal aberrations
Year: 1989
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Not reported
Remark: Polyploid frequencies ranged from 0-3.5% depending on the chemical tested and were not different from control. Specific data for ethyl vanillin were not available. Ethyl vanillin did not affect tubulin polymerization to microtubules.
Test condition: Ethyl vanillin at a single unspecified dose was suspended in olive oil and intraperitoneally injected into groups of 5 male mice. Preliminary chromosomal aberration tests were conducted 6, 24, and 48 hours after treatment. The effect on tubulin polymerization was also examined.
Test substance: m-Methoxy-p-hydroxybenzaldehyde (data for structurally related substance ethyl vanillin)
Conclusion: Ethyl vanillin did not produce chromosomal aberrations.
Reliability: (4) not assignable
The study was reported in an abstract with very limited detail.

30-MAR-2001 (4)

Type: other: Clastogenic assay
Species: other: Mice/BDF1 **Sex:** male
Route of admin.: unspecified
Exposure period: Single dose
Doses: Not reported
Result: ambiguous

Method: other: Micronucleus test
Year: 1989
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Not reported
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: Ethyl vanillin administration did not affect the frequency of micronucleated polychromatic erythrocytes.
Test condition: Ethyl vanillin at a single unspecified dose was suspended in olive oil and intraperitoneally injected into groups of 5 male mice. The frequency of micronucleated polychromatic erythrocytes was determined 24 hours after treatment.
Test substance: m-Methoxy-p-hydroxybenzaldehyde (data for sturcturally related substance ethyl vanillin)
Conclusion: Ethyl vanillin administration did not affect the frequency of micronucleated polychromatic erythrocytes.
Reliability: (4) not assignable
The study was reported in an abstract with very limited detail.
30-MAR-2001 (4)

Type: other: Clastogenic assay
Species: monkey **Sex:** male/female
Strain: NMRI
Route of admin.: unspecified
Exposure period: Two doses at 0 and 24 hours
Doses: 333, 666, or 1,000 mg/kg bw in 3% gum arabic
Result: negative

Method: other: Micronucleus test
Year: 1983
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: The mean number of micronucleated PE/1000 PE at 0, 333, 666, and 1,000 mg/kg bw was 2.5, 2.5, 3.7, and 3.0, respectively.
Test condition: Groups of 10- to 14-week-old NMRI mice were intraperitoneally injected at 0 and 24 hours with 333, 666, or 1,000 mg/kg bw. At 30 hours, the mice were killed and bone marrow smears were prepared using the staining method of Schmid (1976).

Test substance: m-Methoxy-p-hydroxybenzaldehyde (data for sturcturally related substance ethyl vanillin)
Conclusion: Ethyl vanillin did not induce micronuclei in this assay.
Reliability: (2) valid with restrictions
The data were acquired by standard methodology and published in a peer reviewed journal but there was a limited description of the protocol and the results were tabulated.
09-JUL-2001 (35)

Type: other: Clastogenic assay
Species: monkey **Sex:** male/female
Strain: NMRI
Route of admin.: unspecified
Exposure period: Two doses at 0 and 24 hours
Doses: 335, 670, or 1,005 mg/kg bw in olive oil
Result: negative

Method: other: Micronucleus test
Year: 1983
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: The mean number of micronucleated PE/1000 PE at 0, 335, 670, and 1,005 mg/kg bw was 1.9, 2.0, 1.8, and 2.9, respectively
Test condition: Groups of 10- to 14-week-old NMRI mice were intraperitoneally injected at 0 and 24 hours with 335, 670, or 1,005 mg/kg bw. At 30 hours, the mice were killed and bone marrow smears were prepared using the staining method of Schmid (1976).
Test substance: m-Methoxy-p-hydroxybenzaldehyde (data for structurally related substance p-ethoxybenzaldehyde
Conclusion: p-Ethoxybenzaldehyde did not induce micronuclei in this assay.
Reliability: (2) valid with restrictions
The data were acquired by standard methodology and published in a peer reviewed journal but there was a limited description of the protocol and the results were tabulated.
09-JUL-2001 (35)

Type: other: Clastogenic assay
Species: other: Mice/BDF1 **Sex:** male
Route of admin.: unspecified
Exposure period: Up to 21 hours
Doses: 125-500 mg/kg (specific doses not reported)
Result: negative

Method: other: Micronucleus test (Schmid, 1976)
Year: 1988
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Chi-square test, Cochrane-Armitage trend test
Remark: Vanillin by itself did not induce any MN-PCEs. The authors also reported that vanillin administered concurrently with MMC did not alter frequency of MN-PCEs (data and methodology not reported). Treatment with vanillin post MMC injection appeared to reduce the frequency of MN-PCEs, reaching statistical significance at 6 and 9 hours post injection.
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: Vanillin administered after treatment with Mitomycin C was reported to produce a significant reduction in the frequencies of micronucleated polychromatic erythrocytes (MN-PCEs). The frequency of MN-PCEs was reported for 500 mg vanillin/kg bw at time 0, 3, 6, 9, 12, 15, 18, and 21 hours as 4.3, 3.63, 3.23 (p=0.05 from MMC control)), 2.23 (p=0.01 from MMC control), 3.6, 3.57, 4, and 4.47%, respectively. The control groups (not described in methods by authors) of MMC only or vanillin only showed MN-PCE frequencies of 4.5 and 0.13%, respectively.
Test condition: Mice were pretreated with 2 mg mitomycin C (MMC)/kg bw by intraperitoneal injection. At 3, 6, 9, 12, 15, 18 and 21 hours post MMC injection, vanillin doses were administered by gavage. At 24 hours post MMC injection, the bone marrow cells were sampled. In the time-course study, 500 mg vanillin/kg bw suspended in 2% Tween 80 was administered at 7.5 or 9 hours post MMC injection and at 12, 16, 20, 24, 30, 36, 48, and 72 hours post MMC injection, the bone marrow cells were sampled.
Test substance: m-Methoxy-p-hydroxybenzaldehyde
Conclusion: Vanillin suppresses MN-PCE induced by MMC and is most effective at 9 hours after mitomycin-C induction of micronuclei. A reduction of 38-50% was observed.
Reliability: (1) valid without restriction
The data were acquired by standard methodology and published in a peer reviewed journal

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Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** no data
Route of admin.: oral feed
Doses: 50 mM
Result: negative

Method: other: Sex linked recessive lethal mutation assay (Wuergler et al., 1977)
Year: 1983
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Remark: Ethyl vanillin did not increase the number of sex-linked recessive lethal mutations as compared to controls.
Test condition: Flies were exposed to the test compound prepared in a 5% saccharose solution and 2% ethanol and 2% Tween 80 for compounds with poor water solubility. Further details of the methodology were not reported.
Test substance: m-Methoxy-p-hydroxybenzaldehyde (data for structurally related substance ethyl vanillin)
Conclusion: Ethyl vanillin did not induce sex linked recessive lethals in Drosophila melanogaster.
Reliability: (2) valid with restrictions
The data were acquired by standard methodology and published in a peer reviewed journal but there was a limited description of the protocol and the results were tabulated.
30-MAR-2001 (35)

5.8.2 Developmental Toxicity/Teratogenicity

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5.8.3 Toxicity to Reproduction, Other Studies

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IUCLID Data Set

Existing Chemical	ID: 140-11-4
CAS No.	140-11-4
EINECS Name	benzyl acetate
EC No.	205-399-7
TSCA Name	Acetic acid, phenylmethyl ester
Molecular Formula	C9H10O2

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo:	Benzyl Derivatives
Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**
Number of Pages:	**56**
Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = -51.3 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (5)

Value: = -51 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (38)

2.2 Boiling Point

Value: = 213 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (38)

Value: = 213 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (38)

Value: = 215.5 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (5)

Value: = 216 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (9)

2.4 Vapour Pressure

Value: = .13 hPa at 20 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (10)

Value: = .24 hPa at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (6)

Value: = .25 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated/Mean of Antoine & Grain method
Test condition: Calculated based on a measured boiling point of 213 C.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (45)

2.5 Partitio n Coefficient

log Pow: = 1.96 at 25 degree C

Method: other (measured)
GLP: no data

Method: Measured
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (34)

log Pow: = 2.08 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (44)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 3100 mg/l at 25 degree C

Method: other
Year: 1992
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.

16-NOV-2001 (4)

Solubility in: Water
Value: = 1605 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a log Kow = 1.96
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.

16-NOV-2001 (46)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 20.1 hour(s)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (40)

3.1.2 Stability in Water

Type: abiotic

Method: other: Hydrolysis simulated intestinal fluid in vitro hydrolysis
Year: 1977
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: 50% in less than 2 hrs at pH 7.5 in 0.5M phosphate buffer
Test condition: Benzyl acetate (70 µL/L) was incubated with pancreatin at pH 7.5 in 0.5 M phosphate buffer at 37 C for 2 hours. The extent of hydrolysis was determined by GLC.
Conclusion: Benzyl acetate is effectively hydrolyzed at pH 7.5 in intestinal fluid
Reliability: (2) valid with restrictions
16-NOV-2001 (13)

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 20 days at pH 8 and 198 days at pH 7
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (43)

Type: abiotic

Method: other: Hydrolysis in simulated gastric juice in vitro hydrolysis
Year: 1977
GLP: no
Test substance: other TS

Result: half-life = 577 min
Test condition: Benzyl isobutyrate (sat. soln) was incubated with pepsin at pH 1.2 in NaCl/0.1N HCl mixture at 37 C for 4 hours. First order rate constant and t1/2 detemined by GLC.
Test substance: Benzyl acetate (data for structurally related ester, benzyl isobutyrate)
Conclusion: Benzyl isobutyrate is hydrolyzed in simulated gastric juices
Reliability: (2) valid with restrictions
16-NOV-2001 (19)

Type: abiotic

Method: other: Hydrolysis simulated intestinal fluid in vitro hydrolysis
Year: 1977
GLP: no
Test substance: other TS

Result: 100% in less than 2 hrs
Test condition: Benzyl 2-methylbutanoate (40 µL/L) was incubated with pancreatin at pH 7.5 in 0.5 M phosphate buffer at 37 C for 2 hours. The extent of hydrolysis was determined by GLC.
Test substance: Benzyl acetate (data on structurally related ester, benzyl 2-methylbutanoate)
Conclusion: Benzyl 2-methylbutanoate is hydrolyzed at pH 7.5 in intestinal fluid
Reliability: (2) valid with restrictions
16-NOV-2001 (13)

Type: abiotic

Method: other: Hydrolysis in simulated intestinal fluid in vitro hydrolysis
Year: 1977
GLP: no
Test substance: other TS

Result: half-life = 17.8 min
Test condition: Benzyl isobutyrate (sat. soln) was incubated with pancreatin at pH 7.5 in phosphate buffer at 37 C for 4 hours. First order rate constant and t1/2 detemined by GLC.
Test substance: Benzyl acetate (data for structurally related ester, benzyl isobutyrate)
Conclusion: Benzyl isobutyrate is hydrolyzed in simulated intestinal fluid
Reliability: (2) valid with restrictions
16-NOV-2001 (19)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 254000
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (39)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 3.59
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (39)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 11.2
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (39)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.00046
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
16-NOV-2001 (39)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 4.56
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (39)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.79
Aerosol =0.000089% Air =17.6% Fish =0.00035% Sediment =0.14%
Soil =6.15% Suspended Sediment =0.0043% Water =76.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (39)

3.5 Biodegradation

Type: aerobic
Inoculum: other: 10% secondary effluent from sludge from local STP
Contact time: 28 day(s)
Result: other: Mean biodegradation of 100.9% after 28 days (SD 2.5; CI 96.9-104.9) and the authors concluded that the modification produced similar results to the standard protocol but was simpler and more readily replicated.

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 1991
GLP: no data
Test substance: other TS

Result: Degradation % after time: 100.9% at 28 days
Time required for 10% degradation: <2.5 days
Total Degradation: Yes
Test condition: 10 day window criteria: Not reported
10 mg DOC/L at 20 C for 28 days; modification to OECD guidelines was the use of infra-red analyzers to measure CO2; 4 replicates
Test substance: Benzyl acetate (reagent grade)
Conclusion: Benzyl acetate is readily biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines, is published in a peer reviewed journal and results are consistent with chemical structure.
27-APR-2001 (2)

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 0.978; nonlinear - 0.999. Expert survey results: ultimate - 3 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Benzyl acetate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (41)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Acute Fish Toxicity
Species: other: Japanese medaka (Oryzias latipes)
Unit: **Analytical monitoring:** yes

Method: other: Experimental
Year: 1995
GLP: no data
Test substance: other TS

Result: Survival was decreased to 80% at 4.43 mg/L and 0% at the 2 highest doses.
Test condition: Twenty medaka were exposed per concentration. Fish were not fed for 24 hours before or during exposure.
Test substance: Benzyl acetate (>99% purity)
Conclusion: The 96 hour LC50 was reported to be 4.00 mg/L.
Reliability: (1) valid without restriction
Published in a peer reviewed journal. EPA study.
12-MAR-2001 (15)

Type: other: Acute Fish Toxicity
Species: other: Zebra fish (Brachydanio rerio)/Hamilton Buchanan/West-Aquarium
Unit: **Analytical monitoring:** yes

Method: other: E.C. Council Directive 92/69/EEC C.1
Year: 1994
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: No mortalities observed at 5.5, 7.8, and 11 mg/L. 60% mortality (6/10) at 16 mg/L after 96 hours and 100% at 22 mg/L after 72 hrs. Animals (6) at 16 mg/L exhibited sluggish swimming action at 72 hours. Small variations in test conditions were recorded over the 96 hours: Temp, 23-21.7C; [O2] = 7.1-9.3 mg/L; % O2 sat = 94-108%; pH = 7.4-8.0.
Test condition: Zebra fish (10/group) acclimated for 10 weeks were exposed to 4 concentrations of amyl salicylate (5.5, 7.8, 11, 16, and 22 mg/L) under semistatic conditions with daily renewal for 96 hrs. The test substance in synthetic water was treated for 60 sec at 8000 rpm with ultra turrax. Test concentrations were measured at the end of each 24 hr period. Animals were exposed to 12 hours of light in synthetic fresh water. Test solutions were monitored for temperature, O2 concentration, % O2 saturation, and pH. Fish were monitored for behavior and mortality daily. Body weight and length were measured at death or after sacrifice at 96 hours.
Conclusion: Based on nominal test concentrations, LC0 = 11 mg/L, LC100 = 22 mg/L, and LC50 = 15.8 mg/L. Based on measured mean final concentrations, LC0 = 4.6 mg/L, LC100 = 13.7 mg/L, and LC50

= 7.9 mg/L.
Reliability: (1) valid without restriction
Test protocol comparable to OECD semistatic test protocol.
16-NOV-2001 (18)

Type: other: Embryo-larval Test
Species: other: Japanese medaka (Oryzias latipes)
Exposure period: 28 day(s)
Unit: **Analytical monitoring:** yes

Method: other: Experimental
Year: 1995
GLP: no data
Test substance: other TS

Result: Significantly ($p<0.05$) decreased survival reported at concentrations of 1.92 mg/L and higher.
Test condition: Tests were initiated by randomly distributing groups of 60 (0-3 days old) medaka to each of 12 exposure tanks. Fish were placed in dilution water and toxicant dosing pumping increased concentration to intended levels within 3 hrs. Larval survival checked daily.
Test substance: Benzyl acetate (>99% purity)
Conclusion: Estimated maximum acceptable toxicant concentration (MATC) was reported to be between 0.92 and 1.92 mg/L. The chronic NOEC value was calculated to be 1.33 mg/L using the geometric mean of the estimated MATC.
Reliability: (1) valid without restriction
Published in a peer reviewed journal. EPA study.
27-APR-2001 (15)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 96 hour LC50 = 24 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (42)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 48 hour LC50 = 130.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (42)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = 1.9 -

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 96 hour EC50 = 1.9 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (42)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

Type:	LD50
Species:	rat
Strain:	Osborne-Mendel
Sex:	male/female
No. of Animals:	5
Vehicle:	no data
Route of admin.:	other: Gavage
Method:	LD50 calculated by using the Litchfield and Wilcoxon method, dose range is 95 confidence interval
Year:	1964
GLP:	no
Test substance:	as prescribed by 1.1 - 1.4
Remark:	Slope function: 1.6 (95% C.L. 1.1-2.4). Toxic signs were depression. Time of deaths was between 4 hours and 3 days.
Result:	LD50 = 2490 mg/kg bw (95% C.L. 2040-3040) Number of deaths at each dose level: Not reported
Test condition:	Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Reliability:	(2) valid with restrictions This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

30-JUN-2001 (17)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rats showed signs of central nervous system stimulation including: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis).
Result: LD50 = 3690 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 30 rats were used to determine the LD50. Not less than 3 groups of 5 rats were tested. Rats were fasted 24 to 48 hours prior to administration of test substance. Rats were observed for 2 weeks or until death.
Not reported
Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.
30-JUN-2001 (12)

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rabbits showed signs of central nervous system stimulation similar to that reported in rats [ncluding: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis)] followed by a 12- to 24-hour period of prostration prior to death.
Result: LD50 = 2640 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 12 rabbits were used to determine the LD50. Not

less than 3 groups of 3 rabbits were tested. Rabbits were fasted 24 to 48 hours prior to administration of test substance. Rabbits were observed for 2 weeks or until death.
Not reported

Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.

30-JUN-2001 (12)

Type: other: Single dose
Species: rat
Strain: other: F344/N
Sex: male/female
No. of Animals: 5
Vehicle: other:Corn oil
Route of admin.: other: Gavage

Method: NTP
Year: 1986
GLP: yes
Test substance: other TS

Remark: No compound-related clinical signs were observed except for the deaths at the high dose.
Result: Not reported
Number of deaths at each dose level: At 4000 mg/kg bw, 4/5 males and 2/5 females died. No deaths at other doses.
Test condition: Single dose of 250, 500, 1000, 2000 or 4000 mg/kg bw and examined twice daily for clinical signs during a 15-day observation period.
Test substance: Benzyl acetate (food grade, lot no. 9640 ester values were 96.0%, impurities less than 1.0%)
Reliability: (1) valid without restriction
NTP study

30-JUN-2001 (29)

Type: other: Single dose
Species: mouse
Strain: B6C3F1
Sex: male/female
No. of Animals: 5
Vehicle: other:Corn oil
Route of admin.: other: Gavage

Method: NTP
Year: 1986
GLP: yes
Test substance: other TS

Result: Not reported
Number of deaths at each dose level: At 4000 mg/kg bw, all

mice died; at 2000 mg/kg bw 1/5 males and 2/5 females died. No other deaths were reported.

Test condition: Single dose of 250, 500, 1000, 2000 or 4000 mg/kg bw/d and examined twice daily for clinical signs during a 15-day observation period.

Test substance: Benzyl acetate (food grade, lot no. 9640 ester values were 96.0%, impurities less than 1.0%)

Reliability: (1) valid without restriction
NTP study

30-JUN-2001 (29)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 28 days
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0, 20,000, 35,000, or 50,000 ppm (approximately 0, 2, 3.5, or 5 g/kg bw/d) Actual dose: Approximately 0, 2, 3.5, or 5 g/kg bw/d
Control Group: other: basal diet

Method: other: 28-day feeding study
Year: 1995
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Results were analyzed by pairwise comparisons and parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955), or nonparametric multiple comparison methods of Dunn (1964).

Result: LOAEL: 20000 ppm
Toxic response/effects by dose level: All animals died in the highest dose group and 10% died in the 35,000 ppm group. At the 2 highest dose levels, ataxia, convulsions, neuronal necrosis (brain), and lesions in the skeletal muscle, liver, and kidneys were reported. In addition, a statistically significant, dose-related decrease in body weight was

observed in all treated animals. When a glycine supplement (27,000 ppm) was fed in conjunction with the highest dose, mortality was reduced to 10% and there was an absence of behavioral toxicity and reduced neuronal necrosis and hypertrophy.

Test condition: Groups of 35 rats were fed 0, 20,000, 35,000, or 50,000 ppm (approximately 0, 2, 3.5, or 5 g/kg bw/d) for 28 days. A functional observational battery (FOB) test, and histopathology and immunochemistry were conducted. A glycine supplement (27,000 ppm) was fed in conjunction with the highest dose in an additional group.

Test substance: Benzyl acetate (>99% purity)

Conclusion: The authors concluded that adequate levels of glycine were instrumental in reducing the toxicity of benzyl acetate.

Reliability: (2) valid with restrictions
Well documented study. Results considered reliable.

13-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 6 months
Frequency of treatment: daily
Post exposure period: None
Doses: 0.4, 0.8% Actual dose: Approximately 200 or 400 mg/kg bw/day
Control Group: other: diet only

Method: other: induction of pancreatic foci
Year: 1990
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Data comparisons were statistically analyzed using the chi-square test, Student's t-test, linear regression, or an analysis of variance followed by the Newman-Keuls test.
The morphological data provide evidence that benzyl acetate promotes the growth of some acidophilic foci (based on a significant increase in the average size of foci). No increase in the incidence of carcinoma in situ among rats fed benzyl acetate suggest that any promoting influence of benzyl acetate was weak.

Result: LOAEL: 0.8%
NOAEL: 0.4%
Toxic response/effects by dose level: Fewer lesions per cm3 were reported in rats fed benzyl acetate reaching statistical significance in the head sections of high-dose animals. However, the mean diameter and the volume percentage of the foci was significantly greater in benzyl acetate-treated rats than in controls.

Test condition: Male F344 rats (20/group) were injected twice with 30 mg/kg bw of azaserine (carcinogen) at 16 and 23 days of age and fed 0, 0.4 or 0.8% benzyl acetate in the diet (approximately 0, 200, or 400 mg/kg body weight/day, respectively) for a period of 6 months. After 6 months, rats were killed, necropsied, and the pancreas was excised for examination of acinar cell foci in the head (duodenal) and tail (splenic) sections.

Conclusion: The authors concluded that benzyl acetate was a weak promoter, but not an initiator of pancreatic carcinogenesis in the rat.

Reliability: (2) valid with restrictions
Not stated to be conducted under GLP, but well reported.

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 12 months
Frequency of treatment: daily
Post exposure period: None
Doses: 0.4, 0.8% Actual dose: approximately 200 or 400 mg/kg bw/day
Control Group: other: diet only

Method: other: induction of pancreatic foci
Year: 1990
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Data comparisons were statistically analyzed using the chi-square test, Student's t-test, linear regression, or an analysis of variance followed by the Newman-Keuls test.
The authors stated that results were unsatisfactory due to high incidence of chronic renal disease and premature death of animals; however, the results indicatet that benzyl acetate was not a strong promoter of pancreatic carcinogenesis.

Result: Toxic response/effects by dose level: Rats treated with azaserine died prematurely (6-12 months) due to chronic renal disease. Kidneys were reported to be pale, small and finely scarred with tubular atrophy, interstitial fibrosis and chronic inflammation. The severity of renal disease was reported to be greater in rats fed benzyl acetate.

Test condition: Male F344 rats (20/group) were injected twice with 30 mg/kg bw of azaserine (carcinogen) at 16 and 23 days of age and fed 0, 0.4 or 0.8% benzyl acetate in the diet (approximately 0, 200, or 400 mg/kg body weight/day, respectively) for a period of 1 year. After 12 months, rats were killed, necropsied, and the pancreas was excised for examination of acinar cell foci in the head (duodenal) and tail (splenic) sections.

Conclusion: The authors concluded that benzyl acetate was a weak promoter, but not an initiator of pancreatic carcinogenesis in the rat.

Reliability: (2) valid with restrictions
Not stated to be conducted under GLP, but well reported. In addition, results are limited due to premature death of animals.

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily
Post exposure period: None
Doses: 0.8% Actual dose: approximately 400 mg/kg bw/day
Control Group: other: diet only

Method: other: induction of pancreatic foci
Year: 1990
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Data comparisons were statistically analyzed using the chi-square test, Student's t-test, linear regression, or an analysis of variance followed by the Newman-Keuls test.

Result: Toxic response/effects by dose level: Anaplastic changes and desmoplasia (denotes progression to carcinoma in situ) were noted in each group with the largest and most advanced in the control group. A marginal statistically significant increase in the incidence of pancreatic carcinoma in situ among rats fed 0.8% benzyl acetate was reported (percentage of carcinoma in situ in control = 0% and in treated group=8%).

Test condition: Groups of 25 rats were fed AIN-76A diet or diet with 0.8% benzyl acetate. The diet fed from weaning until necropsy at 2 yrs. None of the rats were pretreated with azaserine as was done in 2 co-studies. The pancreas was excised and examined for acinar cell foci and other grossly identified abnormal tissues were fixed for histological study

Conclusion: The authors concluded that benzyl acetate was a weak promoter, but not an initiator of pancreatic carcinogenesis in the rat.

Reliability: (2) valid with restrictions
Not stated to be conducted under GLP, but well reported.

04-MAR-2001 (21)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 13 weeks
Frequency of treatment: daily, ad libitum
Post exposure period: None
Doses: dietary concentrations of 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (approximately 0, 230, 460, 900, 1,750, or 3,900 mg/kg bw/d for males and 0, 240, 480, 930, 1,870, or 4,500 mg/kg bw/d for females) Actual dose: approximately 0, 230,
Control Group: other: basal diet

Method: other: 13-week feeding study
Year: 1993
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 12,500 ppm
NOAEL: 6,250 ppm
Toxic response/effects by dose level: In both sexes, mortality was 90% at the highest dose and comparable with controls at all other dose levels. Final body weights and overall body weight gains were significantly decreased at 12,500 ppm (females; $p<0.05$) and at 25,000 ppm (males and females; $p<0.01$). Secondary to decreased body weights were slight decreases in relative and absolute brain, thymus, kidney, and uterine weights at the high dose. The only statistically significant finding, excluding animals fed the highest dose, in clinical chemistry parameters was lower cholesterol levels in female rats fed 12,500 or 25,000 ppm benzyl acetate. At the highest dose, lesions (i.e., brain necrosis, renal tubule degeneration and regeneration, and degeneration and hyperplasia of the tongue and skeletal muscles) were noted in both sexes. Testicular atrophy was seen in 1 and 2 male rats from the 12,500 (minimal) and 25,000 (marked) ppm groups, respectively. Females fed 25,000 ppm benzyl acetate had an increased volume, surface area, and density of hepatic peroxiso

Test condition: Groups of 10 male and 10 female F344/N rats were fed benzyl acetate in the diet at concentrations of 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (providing doses of approximately 0, 230, 460, 900, 1,750, or 3,900 mg/kg bw/d for males and 0, 240, 480, 930, 1,870, or 4,500 mg/kg bw/d for females, respectively) for 13 weeks. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were weighed and clinically examined weekly and were necropsied at study termination.

Test substance: Benzyl acetate (98% purity)

Conclusion: Benzyl acetate, when fed at up to 460 or 480 mg/kg bw/d for males and females, respectively, for a period of 13 weeks produced no effects in rats.
Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (31)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 103 weeks
Frequency of treatment: daily, ad libitum
Post exposure period: None
Doses: dietary concentrations of 0, 3,000, 6,000, or 12,000 ppm benzyl acetate (approximately 0, 130, 260, or 510 mg/kg bw/d and 0, 145, 290, or 575 mg/kg bw/d for male and female rats, respectively) Actual dose: approximately 0, 130, 260, or 510
Control Group: other: basal diet

Method: other: carcinogenicity assay
Year: 1993
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.
Result: NOAEL: 12,000 ppm
Toxic response/effects by dose level: There was no effect on survival, hematology, or clinical chemistry. Mean body weights and food consumption were slightly reduced in the high-dose groups. No treatment-related changes in the incidence of neoplastic and non-neoplastic lesions were reported.
Test condition: Animals were observed twice daily and body weights were recorded weekly until week 13 and then monthly until the end of the study. At 15 months, blood was collected for hematological and clinical chemistry analyses. At the end of the study, all remaining animals were killed, and organs and tissues were grossly and microscopically examined.
Test substance: Benzyl acetate (98% purity)
Conclusion: NTP concluded, “under conditions of these 2-year feeding studies, there was no evidence of carcinogenic activity of benzyl acetate in male or female F344/N rats receiving 3,000, 6,000, or 12,000 ppm.”
Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (31)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: Lewis or F344
Route of administration: oral feed
Exposure period: 4 months
Frequency of treatment: daily, 5 days per week
Doses: 0.9% benzyl acetate
Control Group: other: diet only

Method: other: induction of pancreatic foci
Year: 1986
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Benzyl acetate did not affect pancreatic growth or induce foci in either rat strain.
Statistical evaluations: Yes. Data comparisons were conducted with X2-test, Student's t-test, linear regression or by an analysis of variance followed by the Newman-Keuls test.
Result: Toxic response/effects by dose level: None
Test condition: Diets were refreshed every other day. The authors estimated that the Lewis rats received about 57% more benzyl acetate than their gavaged counterparts whereas the F344 rats received about 31% more. Rats were necropsied 4 months after weaning and the pancreas was removed, fixed and sectioned after being stained with hematoxylin and eosin. The pancreas was divided into tail and head portions with the tail portion being used for quantitative microscopy. Sections were examined by light microscopy.
Reliability: (2) valid with restrictions
The data were well documented and published in a peer-reviewed journal and therefore were considered reliable.

09-JUL-2001 (22)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 250 or 500 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: carcinogenicity assay
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 500 mg/kg bw/d
NOAEL: 250 mg/kg bw/d
Toxic response/effects by dose level: No significant differences in survival were reported. There was a statistically significant ($p<0.05$) increase in the incidence of preputial gland tumors in males; however, NTP did not associate this with benzyl acetate administration since the combined incidence of adenomas, adenocarcinomas, or carcinomas was not increased. In the pancreas, acinar-cell hyperplasia was reported in all groups of males.
Acinar-cell adenomas in males occurred with a statistically significant ($p<0.01$) positive trend; results of pairwise comparison of controls with high-dose males were also significant ($p<0.01$) with tumors often multiple in rats.
The incidence of the multiple pancreatic acinar cell adenomas in the control, mid- and high-dose male rats was 20 (10/50), 24 (12/50), and 45% (22/49). . This was not observed in females. The incidence of subcutaneous fibromas was significantly increased in low-dose males ($p<0.05$), but trend test results and comparison with the high-dose group with controls was not significant. In females, the

Test condition: Groups of 50 males and 50 females were given 0, 250 or 500 mg benzyl acetate/kg bw/d in corn oil by gavage 5 days/week for 103 weeks. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed 2x daily and weighed weekly for the first 13 weeks and monthly thereafter. At study termination, surviving animals were killed and necropsied. Histopathology was conducted on all animals.

Test substance: Benzyl acetate (food grade, ester values ranged from 99.1-101.3%, impurities were less than 1%)

Conclusion: Under conditions of this study, benzyl acetate increased the incidence of acinar-cell adenomas of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenicity was

found for female F344/N rats.
Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (30)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 14 days
Frequency of treatment: daily
Post exposure period: No
Doses: 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: 14-day gavage study
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).
Result: LOAEL: 2,000 mg/kg bw/d
NOAEL: 1,000 mg/kg bw/d
Toxic response/effects by dose level: All rats in 2 highest dose groups died by day 5. The cecum was redder in 3/5 males and 3/5 females receiving 4,000 mg/kg bw/d than in controls.
Test condition: Groups of 5 males and 5 females were given 0, 250, 500, 1,000, 2,000, or 4,000 mg benzyl acetate/kg bw/d in corn oil by gavage for 14 consecutive days. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed daily and weighed weekly. On day 16, surviving animals were killed and necropsied.
Test substance: Benzyl acetate (food grade, ester values were 96.0%, impurities less than 1.0%)
Conclusion: Based on the increased mortality, the doses for the 13-week study were set at 62.5, 125, 250, 500, and 1,000 mg/kg bw/d.
Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (30)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 62.5, 125, 250, 500, or 1,000 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: 13-week gavage study
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 1,000 mg/kg bw/d
NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: At the highest dose, 2/10 males and 1/10 female died and final mean body weight of males was 12% lower than controls. Also, clinically at the highest dose in males and the 2 highest doses in females, trembling, ataxia, and sluggishness were observed. At necropsy, thickened stomach walls were reported in 2/9 males and 4/10 females.

Test condition: Groups of 10 males and 10 females were given 0, 62.5, 125, 250, 500, or 1,000 mg benzyl acetate/kg bw/d in corn oil by gavage 5 days/week for 13 weeks. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed 2x daily and weighed and clinically examined weekly. On days 92-96, surviving animals were killed and necropsied. Histopathology was conducted on controls, the highest dose group with at least 60% survivors and for all animals dying before study termination.

Test substance: Benzyl acetate (food grade, ester values were 96.0%, impurities less than 1.0%)

Conclusion: Based on these results, doses of 250 and 500 mg/kg bw/d were selected for the 2-yr study.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: Lewis or F344
Route of administration: gavage
Exposure period: 4 months
Frequency of treatment: daily, 5 days per week
Doses: 500 mg/kg bw/day
Control Group: other: deionized water

Method: other: induction of pancreatic foci
Year: 1986
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Benzyl acetate did not affect pancreatic growth or induce foci in either rat strain.
Statistical evaluations: Yes. Data comparisons were conducted with X2-test, Student's t-test, linear regression or by an analysis of variance followed by the Newman-Keuls test.
Result: Toxic response/effects by dose level: None
Test condition: Neat benzyl acetate was administered to rats after they reached 200 g. Rats were necropsied 4 months after weaning and the pancreas was removed, fixed and sectioned after being stained with hematoxylin and eosin. The pancreas was divided into tail and head portions with the tail portion being used for quantitative microscopy. Sections were examined by light microscopy.
Reliability: (2) valid with restrictions
The data were well documented and published in a peer-reviewed journal and therefore were considered reliable.
09-JUL-2001 (22)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: oral feed
Exposure period: 13 weeks
Frequency of treatment: daily, ad libitum
Post exposure period: None
Doses: dietary concentrations of 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (providing doses of approximately 0, 425, 1,000, 2,000, 3,700, or 7,900 mg/kg bw/d for males and 0, 650, 1,280, 2,980, 4,300, or 9,400 mg/kg bw/d for females, respecti
Control Group: other: basal diet

Method: other: 13-week feeding study
Year: 1993
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and

Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 50,000 ppm
NOAEL: none
Toxic response/effects by dose level: One death occurred in one mouse of each sex in the high-dose group. Final body weights and body weight gains were significantly reduced at all doses. Significant differences, secondary to decreased body weights, in absolute and relative brain, kidney, liver, pancreatic, prostatic, seminal vesicle, splenic, testicular and thymus weights were reported in all dose groups. Several female mice from the high-dose group and 1 female each from the 12,500 and 25,000 ppm dose groups had tremors. No other clinical signs were reported. Hematological and clinical chemistry parameters were not affected in either sex. In the high-dose group, brain and liver necrosis were reported in 4 females and 1 male, respectively. No other lesions were reported.

Test condition: Groups of 10 male and 10 female B6C3F1 mice were fed benzyl acetate in the diet at concentrations of 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (providing doses of approximately 0, 425, 1,000, 2,000, 3,700, or 7,900 mg/kg bw/d for males and 0, 650, 1,280, 2,980, 4,300, or 9,400 mg/kg bw/d for females, respectively) for 13 weeks. Animals were individually housed. Water and feed was ad libitum. Animals were weighed and clinically examined weekly and were necropsied at study termination.

Test substance: Benzyl acetate (98% purity)

Conclusion: NTP (1993) reported that the administration of benzyl acetate to mice produced effects on final body weight and weight gain at all dose levels tested. It was also noted that mice were not as sensitive as rats also tested by the NTP under similar conditions.

Reliability: (1) valid without restriction
NTP study

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: oral feed
Exposure period: 103 weeks
Frequency of treatment: daily, ad libitum
Post exposure period: None
Doses: dietary concentrations of 0, 330, 1,000, or 3,000 ppm (approximately and 0, 35, 110, or 345 mg/kg bw/d and 0, 40, 130, or 375 mg/kg bw/d for male and female mice, respectively). Actual dose: approximately 0, 35, 110, or 345 mg/kg bw/d and 0
Control Group: other: basal diet

Method: other: carcinogencity assay
Year: 1993
GLP: yes
Test substance: other TS

Remark: NTP considered that the nasal lesions were likely a result of irritation from benzyl acetate vapors
Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 3,000 ppm
Toxic response/effects by dose level: Survival was similar to controls with the exception of the high-dose females, which was significantly higher than the control group. Mean body weights were generally 2 to 14% lower than controls (statistical significance not reported). There were no biologically significant changes in hematology or clinical chemistry parameters. Notable was the lack of an increase in neoplasm incidences, particularly hepatocellular and forestomach neoplasm, as was reported in the gavage studies. A dose-related increase in the incidence or severity of non-neoplastic nasal lesions (i.e., mucosa atrophy and degeneration, cystic hyperplasia of the submucosal gland, and luminal exudates and pigmentation of the mucosal epithelium) was reported.

Test condition: B6C3F1 mice were fed benzyl acetate in the diet at concentrations of 0, 330, 1,000, or 3,000 ppm (approximately 0, 35, 110, or 345 mg/kg bw/d and 0, 40, 130, or 375 mg/kg bw/d for male and female mice, respectively). Animals were observed twice daily and body weights were recorded weekly until week 13 and then monthly until the end of the study. At 15 months, blood was collected for hematological and clinical chemistry analyses. At the end of the study, all remaining animals were killed, and organs and tissues were grossly and microscopically examined.

Test substance: Benzyl acetate (98% purity)

Conclusion: NTP concluded that “there was no evidence of carcinogenic activity of benzyl acetate in male or female B6C3F1 mice” under the conditions of this study.

Reliability: (1) valid without restriction
NTP study
04-MAR-2001 (31)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 500, or 1,000 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: carcinogenicity assay
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 1,000 mg/kg bw/d
NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: The survival of high-dose females was significantly increased (p=0.005) when compared with controls (control, 15/50; low-dose, 18/50; high-dose, 30/50). There were no adverse clinical signs. Body weights treated female mice were slightly higher than controls after week 20. The incidence of hepatocellular adenomas was increased in males (0, 10, and 26%, respectively) and females (0, 0, and 12%, respectively) and reached statistical significance at the highest dose (males, $p<0.001$; females, $p<0.05$). There was no effect on the incidence of hepatocellular carcinomas (males: 10/50, 14/49, 12/50 and females: 1/50, 0/50, 4/50). Also in the high-dose groups of both sexes, there was a statistically significant ($p<0.005$) increase in the occurrence of forestomach hyperplasia. Although there was a positive trend for an increased incidence of combined forestomach squamous cell papillomas and carcinomas in both sexes, it did not reach statistical significance.

Test condition: Groups of 10 males and 10 females were given 0, 500, or 1,000 mg benzyl acetate/kg bw/d in corn oil by gavage 5 days/week for 103 weeks. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed 2x daily and weighed weekly for the first 13 weeks and monthly thereafter. At study termination, surviving animals were killed and necropsied. Histopathology was conducted on all animals.

Test substance: Benzyl acetate (food grade, ester values ranged from 99.1-101.3%, impurities were less than 1%)

Conclusion: Under conditions of this study, there was some evidence of

carcinogenicity in male and female B6C3F1 mice in that benzyl acetate caused increased incidences of hepatocellular adenomas and squamous cell neoplasms of the forestomach.

Reliability: (1) valid without restriction
NTP study

04-MAR-2001 (30)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 14 days
Frequency of treatment: daily
Post exposure period: No
Doses: 0, 125, 250, 500, 1,000, or 2,000 mg/kg bw/d
Control Group: other: corn oil (vehicle)

Method: other: 14-day gavage study
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), and Tarone (1975).

Result: LOAEL: 1,000 mg/kg bw/d
NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: All animals in the highest dose died by day 3. Clinical signs of ruffled fur and ataxia were reported in high-dose males and labored breathing and hperactivity in high-dose females. Roughened mucosa of the stomach was noted in 2/5 males and 5/5 females of the high-dose group and in 1/5 females receiving 1,000 mg/kg bw/d.

Test condition: Groups of 5 males and 5 females were given 0, 125, 250, 500, 1,000, or 2,000 mg benzyl acetate/kg bw/d in corn oil by gavage for 14 consecutive days. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed daily and weighed weekly. On day 16, surviving animals were killed and necropsied.

Test substance: Benzyl acetate (food grade, ester values were 96.0%, impurities less than 1.0%)

Conclusion: Based on the mortality data, the doses selected for the 13-week study were 62.5, 125, 250, 500 and 1,000 mg/kg bw/d for males and 125, 250, 500, 1,000, and 2,000 mg/kg bw/d for females.

Reliability: (1) valid without restriction
NTP study

04-MAR-2001 (30)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 weeks
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0, 125, 250, 500, 1,000, or 2,000 mg/kg bw/d (females); 0, 62.5, 125, 250, 500, or 1,000 mg/kg bw/d (males)
Control Group: other: corn oil (vehicle)

Method: other: NTP
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Methodology of Kaplan and Meier (1958), Cox (1972), Mantel -Haenszel (1959), and Tarone (1975). The Fisher exact/Cochran-Armitage trend analysis was also used.

Result: LOAEL: 1,000 mg/kg bw/d
NOAEL: 500 mg/kg bw/d
Toxic response/effects by dose level: In the high-dose animals, 8/10 females died (one death due to gavage error) and the mice exhibited trembling, inactivity, labored breathing and depressed body temperature. There was no compound-related mortality in male mice. No compound-related gross or microscopic pathological effects were reported in any of the treated animals.

Test condition: Groups of 10 males were given 0, 62.5, 125, 250, 500, or 1,000 mg benzyl acetate/kg bw/d and and 10 females were given 0, 125, 250, 500, 1,000, or 2,000 mg benzyl acetate/kg bw/d in corn oil by gavage 5 days/week for 13 weeks. Animals were grouped 5 per cage. Water and feed was ad libitum. Animals were observed 2x daily and weighed and clinically examined weekly. On days 92-96, surviving animals were killed and necropsied. Histopathology was conducted on controls, the highest dose group with at least 60% survivors and for all animals dying before study termination.

Test substance: Benzyl acetate (food grade, ester values were 96.0%, impurities less than 1.0%)

Conclusion: Based on these results, doses of 500 and 1000 mg/kg bw/d were selected for the 2-yr study

Reliability: (1) valid without restriction
NTP study

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 2-300 nmol/plate
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Ames assay
Year: 1986
GLP: no
Test substance: other TS

Result: Benzyl acetate did not increase the number of revertants.
Test condition: Benzyl acetate was dissolved in DMSO (<0.02ml/plate) which was also used as a solvent control. After a 48-hour incubation at 37 C, the number of revertants was counted. Toxicity was determined from the number of surviving colonies. Two experiments were conducted in triplicate. Metabolic activation: none
Test substance: Benzyl acetate (>99% purity)
Conclusion: Benzyl acetate was non mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal. Results presented in tabular form and some detail of methodology was not presented.
01-APR-2001 (33)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster lung fibroblast cell line
Concentration: 0-2.4 mg/ml
Cytotoxic Concentration: 0.9 mg/ml
Metabolic activation: with and without
Result: positive

Method: other: Chromosomal aberrations Based on historical controls, when the percent of aberrations was greater than 10, said to be 'positive'.
Year: 1996
GLP: no
Test substance: other TS

Remark: The authors considered benzyl acetate to be negative in this assay because it did not induce aberrations in any of the other treatment regimes and the authors considered the concentration used in this study was sufficient.
Result: Benzyl acetate marginally induced structural aberrations which were predominantly chromatid exchanges at the 24-hour highest concentration treatment without S9. Precipitate occurred at concentrations of 0.6 mg/ml and higher.
Test condition: Benzyl acetate was dissolved in DMSO which was also used as the negative control. Mitomycin C and cyclophosphamide (with or without S9) were used as positive controls. Cells

were exposed to benzyl acetate for 24 or 48 hours without S9 mix or for 6 hours in with or without S9 mix. After the 6 hour treatments, the cells were cultured in fresh medium for another 18 hours. All slides were coded and 100 well-spread metaphases were analyzed for each dose group. Metabolic activation: with and without rat liver microsome fraction S9 and cofactors

Test substance: Benzyl acetate (>98% purity)

Conclusion: Benzyl acetate did not induce in chromosomal aberrations in this study.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.

01-APR-2001 (23)

Type: Ames test

System of testing: bacterial Salmonella typhimurium TA98, TA100, TA1535, TA1537

Concentration: 0, 33, 100, 333, 1000, 3333, or 10000 ugl/plate

Cytotoxic Concentration: not reported

Metabolic activation: with

Result: positive

Method: other: Ames assay (Haworth et al., 1983)

Year: 1986

GLP: yes

Test substance: other TS

Result: Benzyl acetate produced no increased incidence of mutation as compared to the vehicle controls, either with or without S9 mix.

Test condition: Metabolic activation: rat liver microsome fraction S9
Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98), and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains without S9. 2-Aminoanthracene was used with all strains incubated with S9. Solvent controls were also prepared concurrently. Preliminary tests were conducted to assess the cytotoxicity of the test compound and establish suitable concentrations for testing. At least 5 concentrations of the test chemicals (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 C for 48 hours. A test chemical was considered “mutagenic” if there was a dose-related, reproducible increase in the number of revertants over background (not required to be 2-fold increase), “non mutagenic” if there was no increase, and “questionable” if there was no clear reproducible dose-related increase or “when the response was of insufficient magnitude to support a determination of mutagenicity”.

Test substance: Benzyl acetate (99.1% purity)

Conclusion: Benzyl acetate was non-mutagenic.

Reliability: (1) valid without restriction
NTP study

04-APR-2001 (28)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA98, TA100
Concentration: 3 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Spot tests are less sensitive than quantitative experiments. Also, the results from strain TA100 are difficult to interpret because the high growth background (150-200 colonies per plate); however, spot tests provide a good "screening" method for large numbers of chemicals.
Result: Benzyl acetate produced a negative response in this assay.
Test condition: For each experiment viable count was determined, the number of spontaneous revertants was measured, the presence of the rfa-mutation was determined by crystal violet inhibition, the presence of the plasmid pKM 101 in strains TA98 and TA100 was determined by resistance to ampicillin, and the response to positive controls N-methyl-N-nitrosoquanidin (without metabolic activation) and 2-aminoanthracene (with activation) was determined. Spectroscopic-grade ethanol was used as the solvent. The test substance was tested at 3 umol/plate in TA98, TA100, TA1535, and TA1537 with or without S9. If there was no background lawn of bacteria, the tests were redone using lower concentrations. Uncertain results prompted the conduction of the tests at 4 concentration levels (0.03, 0.3, 3 and 30 umol/plate). Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats
Conclusion: Benzyl acetate is non-mutagenic in the Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 with or without S9.
Reliability: (1) valid without restriction
Study is published in a peer reviewed journal with adequate description and follows standard procedures.

15-MAY-2001 (7)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 160-1600 ug/ml (without S9); 500-5000 ug/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Chromosomal aberrations (Galloway et al., 1985) Linear regression analysis, binomial sampling assumption, and Dunnett's method for multiple dose comparison were used to evaluate the data.
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Benzyl acetate was negative for chromosomal aberration induction.
Result: Slight increase in chromosomal aberrations were observed but these were found to be not statistically significant.
Test condition: Metabolic activation: with and without rat liver microsome fraction S9 and cofactors
Positive controls consisted of treatment with mitomycin C, triethylenemelamine, or cyclophosphamide and negative controls were solvents used to dissolve the test chemical. Tests were carried out with (2-hr test substance exposure) or without S9 (exposure throughout incubation) activation (male Sprague-Dawley rat hepatocytes induced with Aroclor 1254). Cells were harvested 8-12 hours after the beginning of the treatment, yielding cells in mitosis. 100 cells were scored from each of the three highest dose groups having sufficient metaphases for analysis and from positive and solvent controls. All types of aberrations were recorded and they were grouped as either "simple", "complex", or "other" and "total".
Conclusion: Benzyl acetate did not induce chromosomal aberrations in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
01-APR-2001 (11)

Type: Sister chromatid exchange assay
System of testing: non bacterial Chinese hamster ovary cells
Concentration: 50-500 ug/ml (without S9); 500-5000 ug/ml (with S9)
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: negative

Method: other: Sister chromatid exchange (Galloway et al., 1985)
Linear regression analysis was used to test trend. A 20% absolute increase over the control, at each dose, was considered to be significant.
Year: 1987
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Result: No induction of sister chromatid exchange.
Test condition: Chemical treatment periods were approximately 25 hours without S9 (after 2 hours of test chemical exposure, 5-bromodeoxyuridine was added) and 2 hours with S9 (after which 5-bromodexoyuridine was added). After treatment with hypotonic KCl, cells were fixed, stained and examined with fluorescent microscopy. 50 cells per dose were scored from the three highest concentrations when sufficient M2 cells were available, from the control groups.
Metabolic activation: with and without rat liver microsome fraction S9 and cofactors
Conclusion: Benzyl acetate did not induce sister chromatid exchange.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
01-APR-2001 (11)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 5, 50, 500, 2000, or 5000 ug/plate
Cytotoxic Concentration: >2000 ug/plate
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1988
GLP: no
Test substance: other TS

Remark: Benzyl acetate was slightly toxic at the concentrations tested.
Result: Benzyl acetate produced no increase in reverse mutations.
Test condition: Benzyl acetate in isopropyl alcohol was irradiated with a UV lamp or natural sunlight with or without photosensitizer (acetophenone or benzophenone). Reaction mixtures were prepared for incubation with bacteria with or without S9. Three replicates were performed.
Metabolic activation: S9 prepared from livers of male Sprague-Dawley rats
Test substance: Benzyl acetate (reagent grade)
Conclusion: The authors concluded that the photodegradation of benzyl acetate did not increase mutagenic activity.
Reliability: (2) valid with restrictions
02-APR-2001 (35)

Type: other: forward mutation test
System of testing: non bacterial TK6 human lymphoblasts
Concentration: 0-1500 ug/ml
Cytotoxic Concentration: 500 ug/ml
Metabolic activation: with
Result: positive

Method: other: Human lymphoblast assay The data were evaluated using the Poisson distribution and the Dunnett's t-test (one-tail).
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate induced a mutagenic response only in the presence of S9.
Test condition: Metabolic activation: with rat liver microsome fraction S9 from Aroclor induced rats
Solvent controls and positive controls with S9 (benzo[a]pyrene) and without S9 (4-nitroquinoline-N-oxide) were used. Preliminary tests were conducted to assess the cytotoxicity of the test compound and establish suitable concentrations for testing. It was intended that the highest test concentration should reduce relative suspension growth to 10-20% of that in controls. Cultured cells were exposed to multiple concentrations (in duplicate or triplicate) of benzyl acetate for 20 hours (without S9) or 3 hours (with S9). To terminate exposure, cells were centrifuged and resuspended, and then were cultured another 3 days (phenotypic expression period). Cells were plated in microtiter plates with or without trifluorothymidine (selective agent) and after a 12-day incubation the plates were scored and the number of positive wells and total number of wells were recorded. Plating efficiency and mutant fraction were calculated. A chemical was only considered mutagenic if the mean mutant fraction was greater than the 99% upper confidence limit of all historical ne
Conclusion: Metabolically activated benzyl acetate was mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.

04-APR-2001 (3)

Type: Mouse lymphoma assay
System of testing: non bacterial L5178Y mouse lymphoma cells
Concentration: 0-1500 ug/ml
Cytotoxic Concentration: 500 ug/ml
Metabolic activation: with
Result: positive

Method: other: Mouse lymphoma assay The data were evaluated using a trend test and a pairwise comparison of the variance of the mutant fraction (treated) against that of solvent controls.
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate induced a mutagenic response only in the presence of S9.
Test condition: Metabolic activation: with rat liver microsome fraction S9 from Aroclor induced rats
Solvent controls and positive controls with S9 (3-methylcholanthrene) and without S9 (ethyl methanesulfonate or methyl methanesulfonate) were used. Cell cultures were exposed to multiple concentrations (usually in triplicate) of benzyl acetate with or without S9 for 4 hours at 37 C after which they were washed and resuspended for the expression and growth period of 2 days. Cultures were seeded with or without trifluorothymidine (selective agent) to determine cloning efficiency. Cells were incubated for 11-12 days at 37 C. Colonies were electronically counted. The mutant fraction was calculated and the relative total growth was measured. A response was considered “negative” if there was no trend or peak response, “questionable” if there was only a trend response or only a single dose increase with no trend, and “positive” if $p<0.05$ for at least one of the 3 highest concentration sets and if the trend was significant ($p<0.05$) and if it was repeated.
Conclusion: Metabolically activated benzyl acetate was mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.

Type: other: aneuploidy assay
System of testing: yeast Saccharomyces cerevisiae D61.M
Concentration: 520, 676, 831, 1,039, 1,298, or 1,817 ug/ml
Metabolic activation: no data
Result: positive

Year: 1989
GLP: no
Test substance: other TS

Result: Benzyl acetate did not induce chromosome loss, unless cytotoxic levels were reached.
Test condition: Concentrations were considered cytotoxic when "the titer of colony-forming units at the end of the treatment was lower than at the time when the test chemicals were added". Mitotic crossing-over, mitotic gene conversion, point mutation or deletion were indicated by the frequency of red resistant colonies. White resistant colonies were classified as requiring leucine (indicative of chromosome loss), not requiring leucine (indicating other genetic changes), and respiratory-deficient. White colonies classified as "requiring leucine" were used to calculate chromosomal loss.
Test substance: Benzyl acetate (>99% purity)
Conclusion: Benzyl acetate was non genotoxic in this assay.
Reliability: (1) valid without restriction
The study was conducted under the direction of a recognized research institute and was published in a peer-reviewed journal.
04-JUL-2001 (49)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: maximum concentration = 20 ul/disk
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1986
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate was reported to have a inhibition zone difference of greater than or equal to 4 mm and was considered to be weakly positive.
Conclusion: Benzyl acetate was reported to have weak DNA damaging potential.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.
04-JUL-2001 (47)

Type: other: mutation test
System of testing: bacterial E. coli WP2 uvrA (trp-)
Concentration: 0.25-2.0 mg/plate
Metabolic activation: no data
Result: positive

Year: 1986
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate was considered negative since the ratio was 1.4.
Test condition: The results were considered positive if the ratio of maximal revertants to spontaneous revertants was <2.
Conclusion: Benzyl acetate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.
04-JUL-2001 (47)

Type: other: antimutation test
System of testing: bacterial E. coli WP2 uvrA (trp-)
Concentration: 2.0-8.0 mg/ml
Metabolic activation: with
Result: positive

Year: 1986
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate was considered negative since the ratio was 98%.
Test condition: Metabolic activation: induction with AF-2
The results were considered positive if the ratio of minimal revertants to AF-2-induced revertants was <50%.
Conclusion: Benzyl acetate showed no activity in this assay.
Reliability: (2) valid with restrictions
The methodology used followed standard protocols and although most of the study was in Japanese, the tables clearly documented the results.
04-JUL-2001 (47)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 21 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Conclusion: Benzyl acetate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.
04-JUL-2001 (32)

Type: Unscheduled DNA synthesis
System of testing: non bacterial Male F344 rat hepatocyte
Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Unscheduled DNA synthesis
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Benzyl acetate yielded less than 0.0 net grains/nucleus.
Test condition: Metabolic activation: not reported
Treated cells were incubated with 3H-TdR (incubation period not stated) and UDS was measured by quantitative autoradiography as net grains/nucleus. Positive controls used were 2-acetylaminofluorene and dimethylnitrosamine.
Conclusion: Benzyl acetate did not damage DNA in this assay.
Reliability: (2) valid with restrictions
The data were reported in a brief abstract with limited description; however, the research group has published similar study protocols in peer-reviewed journals showing adherence to standard methodologies. Therefore the data were considered reliable.
08-JUL-2001 (24)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Clastogenic assay
Species: other: Mouse/B6C3F1 **Sex:** male
Route of admin.: unspecified
Exposure period: 3 days
Doses: 0, 312, 625, or 1250 mg/kg bw/d (dose volume=0.4 ml/mouse)
Result: negative

Method: other: Micronucleus test
Year: 1993
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Appropriate statistical evaluations: Yes. Statistical significance was determined by a one-tailed trend test, ANOVA, and pairwise comparisons.
Remark: The percent PCE at 0, 312, 625, or 1250 mg/kg bw/d was 69.9, 65.8, 64.3, or 60.7, respectively. Benzyl acetate was negative in this assay.
Result: Effect on mitotic index or PCE/NCE ratio by dose level and sex: The number of MN-PCE/1000 PCE scored for 0, 312, 625, and 1,250 mg/kg bw/d was 3.00, 2.90, 3.20, and 1.80, respectively.
Test condition: Groups of 5-7 male B6C3F1 mice were intraperitoneally injected with 0, 312, 625, or 1250 mg benzyl acetate/kg bw/d in corn oil. Positive controls were administered DMBA in corn oil. Twenty-four hours after treatment, mice were killed and bone marrow smears prepared by fixing in absolute ethanol and staining with acridine orange. The slides were evaluated for number of MN-PCE among 2000 PCE and for the percentage of PCE among 200 erythrocytes.
Conclusion: Benzyl acetate did not induce micronuclei when tested at repeated doses up to 1,250 mg/kg bw/d.
Reliability: (1) valid without restriction
Published in peer reviewed journal and standard protocols were used.

30-MAR-2001 (36)

Type: other: DNA damage
Species: other: Rat/Fischer 344 **Sex:** male
Route of admin.: unspecified
Exposure period: Single dose
Doses: 150, 500, or 1,500 mg/kg bw benzyl acetate
Result: negative

Method: other: Alkaline elution analysis of DNA (Curphey et al., 1987)
Year: 1990
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Appropriate statistical evaluations: Data comparisons were statistically analyzed using the chi-square test, Student's t-test, linear regression, or an analysis of variance followed by the Newman-Keuls test.
Remark: Pancreatic cells removed from benzyl acetate-treated rats showed no evidence of DNA and there was no difference in the alkaline elution profiles of pancreatic DNA from treated or control animals.
Test condition: Groups of one rat were administered a single dose of 0, 150, 500, or 1,500 mg benzyl acetate/kg bw by intraperitoneal injection. After 1 hour, the rats were killed and nuclei were isolated from pancreas. DNA damage was assayed by alkaline elution and duplicate filters used for each pancreas.
Conclusion: No evidence of DNA damage in rats injected with benzyl acetate at up to 1,500 mg/kg bw.
Reliability: (2) valid with restrictions
Not stated to be conducted under GLP, but data were acquired by standard methodology and published in a peer reviewed journal.
09-JUL-2001 (20)

Type: other: DNA damage
Species: other: Rat/Fischer 344 **Sex:** male
Route of admin.: unspecified
Exposure period: Single bolus dose
Doses: 50, 200 or 1000 mg/kg bw
Result: negative

Method: other: Unscheduled DNA synthesis
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Appropriate statistical evaluations: Not reported
Remark: Benzyl acetate produced a negative number for the net grains/nucleus and the percentage of cells undergoing repair was <10% and therefore was considered not to induce UDS in rats.
Test condition: Groups of male Fischer 344 rats (number not specified) were administered a single bolus dose of 50, 200, or 1000 mg benzyl acetate/kg bw in corn oil by gavage. Hepatocyte

cultures were prepared by perfusing the liver taken from the rats at 2 or 12 hours. Quantitative autoradiographic grain counting was used to measure UDS. The net grains/nucleus were determined by subtracting the highest of 2 nuclear-sized areas over the cytoplasm and adjacent to the nucleus from the nuclear count. The percent of cells exhibiting 5 or more net grains/nucleus were considered the percentage of cells undergoing repair. For each rat and concentration, 3 slides were scored. A result was considered negative if the net grains/nucleus of all dose groups was a negative number and the percentage of cells undergoing repair was <10%. Results were considered equivocal if net grains/nucleus of all dose groups was a negative number but the percentage of cells undergoing repair was >10%.

Conclusion: Benzyl acetate does not induce unscheduled DNA synthesis in rats.

Reliability: (1) valid without restriction
Part of the NTP testing program.

30-MAR-2001 (25)

Type: other: DNA damage
Species: other: Rat/F344 **Sex:** male
Route of admin.: unspecified
Exposure period: Not reported
Doses: Up to 1,000 mg/kg bw
Result: negative

Method: other: Unscheduled DNA synthesis
Year: 1984
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Administration of benzyl acetate resulted in a NG of less than 0.

Test condition: After treatment, the rat was killed and the pancreas was removed, minced in a collagenase solution, and digested with dipase to isolate pancreatic cells. Cells were incubated with 3H-thymidine for 18-22 hours, following which, UDS was measured [net grains/nucleus (NG)] by quantitative autoradiography. If NG>3, cells were considered in repair.

Conclusion: Benzyl acetate did not damage DNA in this assay.

Reliability: (4) not assignable
The data were presented in a brief abstract and indicated that a standard protocol had been modified to measure UDS in pancreatic cells.

09-JUL-2001 (37)

Type: other: Lethal mutation test
Species: other: Drosophila melanogaster **Sex:** male
Route of admin.: unspecified
Exposure period: 48 to 72 hours
Doses: 300 ppm (feed); 20000 ppm (injection)
Result: ambiguous

Method: other: Sex-linked recessive lethal (SLRL) assay
Year: 1994
GLP: no
Test substance: other TS

Method: Appropriate statistical evaluations: Yes. Poisson analysis and binomial distribution.
Remark: No induction of recessive lethals with benzyl acetate through feeding or injection.
Test condition: Benzyl acetate was applied to 2 or 3 glass fibre filter disks in a 5% sucrose solution in glass vial. Solutions were renewed at 24 and 48 hours and males (Canton-S) were exposed for 72 hours after which males were mated with 3 virgin Basc females and transferred to fresh females every 2-3 days for a total production of 3 broods. No more than 100 F1 females were mated over the 3 broods from any P1 male in order to avoid recovery of multiple lethals from 1 male. If the number of wild-type males was 0, 1, or <5% of the Basc males, then the F2 cultures were scored as presumptive lethals. If feed exposure was non mutagenic, males were injected with benzyl acetate in a 0.7% NaCl solution. After 24 hours, surviving males were mated. Controls received solutions without benzyl acetate.
Test substance: Benzyl acetate (99.1% purity)
Conclusion: Benzyl acetate was non mutagenic in the SLRL assay.
Reliability: (1) valid without restriction
NTP study

30-MAR-2001 (8)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male
Strain: Wistar
Route of administration: gavage
Exposure period: Gestation days 6-15
Frequency of treatment: Daily
Duration of test: 15 days
Doses: 0, 10, 100, 500, or 1,000 mg/kg bw/d Actual dose: 0, 10, 100, 500, or 1,000 mg/kg bw/d
Control Group: other: The control group was described as "subjected to no oral administration".
Result: NOAEL Developmental Toxicity: 100 mg/kg bw/d LOAEL Developmental Toxicity: 500 mg/kg bw/d

Year: 1993
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results were analyzed using Bartlett's test, test method of Kruskal-Wallis, Dunnett's multiple comparison testing method or Scheffe's multiple comparison testing method
The authors suggested that the skeletal malformations were related to the significant decrease in fetal body weight. No increase in intrauterine death or external variations was noted at any dose level. No adverse effects were seen at or below 500 mg/kg bw/d.

Result: Fetal data with dose level: There was no reported difference in the number of corpora lutea, implantations, live/dead fetuses, or resorptions, implantation ratio, sex ratio, or placenta weight in any treatment group. At the highest dose level, fetal body weight was significantly decreased ($p<0.05$), but was significantly increased in the 2 lowest dose groups ($p<0.05$). There was a statistically significant increase in the combined incidence of organ variations (i.e., slight dilatation of the lateral ventricle and renal pelvis, and presence of levo-umbilical artery) in animals from the 2 highest dose groups. The only skeletal malformation (fused ribs) was in one fetus of the high-dose group, which did not increase the incidence of skeletal malformations compared to controls. Skeletal variations (i.e., wavy ribs, dumbbell shaped vertebrae, absence/splitting of thoracic vertebrae, presence of lumbar ribs and degree of ossification) were statistically increased in the high-dose group.
Maternal data with dose level: No effects on parameters examined.
NOAEL Maternal Toxicity: >1000 mg/kg bw/d

Test condition: Groups of 20 pregnant Wistar rats were administered 0, 10, 100, 500, or 1,000 mg benzyl acetate/kg bw/d by gavage during gestation days 6-15. On day 20 of gestation (term), pregnancies were terminated and the fetuses were examined for intrauterine death, and internal, external and skeletal

malformations. Maternal parameters included mortality, body weight, food consumption, and clinical and gross examinations.

Test substance: Benzyl acetate (99% purity)

Conclusion: The results of this study show no evidence of teratogenic effects for benzyl acetate.

Reliability: (2) valid with restrictions
Study was reported in Japanese with a thorough English translation that describes a well-conducted teratology/developmental study, but does not indicate if it was conducted under GLP.

11-MAR-2001 (16)

Species: mouse **Sex:** male
Strain: other: CD-1
Route of administration: gavage
Exposure period: Gestation days 6-13
Frequency of treatment: Daily
Duration of test: up to day 3 postpartum
Doses: 750 mg/kg bw/d
Control Group: other: A control group of 50 mice gavaged with distilled water only.

Method: other (calculated): Postnatal mouse screening test (Chernoff and Kavlock, 1982), Developmental
Year: 1983
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results were analyzed by ANOVA, Kruskal-Wallis H test, and ANCOVA.

Result: Fetal data with dose level: Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival.
Maternal data with dose level: Clinical signs of maternal toxicity included hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnea, swollen or cyanotic abdomen, and piloerection. There was no significant difference in maternal body weight measured on days 4 and 7 of gestation between treated and control animals. However, statistically significant decreases were observed in treated females on gestation day 18 and day 3 postpartum. Maternal body weight gain during days 7-18 of gestation was also significantly lower than that of controls. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated.

Test condition: 750 mg benzyl alcohol/kg bw/day was administered by gavage to 50 mice on gestation days 6-13. A control group of 50 animals were given distilled water only. Maternal body

weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 postpartum, litter weight change between days 1 and 3 postpartum, and pup survival on days 1 and 3 postpartum were recorded. Clinical signs of maternal toxicity were reported.

Test substance: Benzyl acetate (data for structurally related substance benzyl alcohol)

Conclusion: Although the authors concluded that benzyl alcohol was a potential reproductive hazard, the effects observed were in conjunction with significant maternal toxicity.

Reliability: (2) valid with restrictions
NIOSH study. Audited and found to follow SOPs, but not reported to be GLP.

13-MAR-2001 (14)

Species: mouse **Sex:** male
Strain: other: CD-1
Route of administration: gavage
Exposure period: Gestation days 6-15
Frequency of treatment: Daily
Duration of test: up to day 3 postpartum
Doses: 0 or 550 mg/kg bw/d in corn oil
Control Group: other: The control group received corn oil only.
Result: NOAEL Developmental Toxicity: >550 mg/kg bw/d

Method: other (calculated): Postnatal mouse screening test (Chernoff and Kavlock, 1982; Waters, 1983), Developmental
Year: 1986
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Results were analyzed by Bartlett's test, F-test, ANOVA, Fischer's exact test, Mann-Whitney U-Test

Result: Fetal data with dose level: All parameters tested, including gestation index were statistically similar for the treated and control animals.
Maternal data with dose level: All parameters tested, including average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals.
NOAEL Maternal Toxicity: >550 mg/kg bw/d

Test condition: In a preliminary dose range-finding study, groups of 4 CD-1 mice were administered 200, 380, 720, 1,370, or 2,605 mg benzyl alcohol/kg bw/d by gavage during gestation days 6-15. No control group was used. All animals died at the highest dose and 2 animals died at the second highest dose. At 720 mg/kg bw/d, there was no signs of toxicity except for reduced body weight. Based on these results, a dose level of 550 mg/kg bw/d was selected for a teratology study. In the teratology study, groups of 50 pregnant CD-1 mice were administered 0 or 550 mg benzyl alcohol/kg bw/d in corn oil by gavage during gestation days 6-15. Body weight, clinical observations, and mortality were recorded daily throughout

treatment and up to 3 days postpartum.
Test substance: Benzyl acetate (data for structurally related substance benzyl alcohol)
Conclusion: In this assay, benzyl alcohol did not produce evidence of developmental toxicity.
Reliability: (1) valid without restriction
Audited study conducted under GLP.
13-MAR-2001 (48)

5.8.3 Toxicity to Reproduction, Other Studies

Type: other:
In Vitro/in vivo: In vivo
Species: mouse
Strain: B6C3F1 **Sex:** male
Route of administration: other: Diet
Exposure period: Premating exposure, Females: None Premating exposure, Males: None
Frequency of treatment: Daily
Duration of test: 13 weeks
Doses: 3,130, 6,250, 12,500, 25,000, or 50,000 ppm
Control Group: other: Basal diet

Method: other: NTP 13-week study with sperm morphology and vaginal cytology examinations (SMVCEs)
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: In male mice, terminal body weights were decreased (specific dose levels not reported). Absolute weights of the right cauda, epididymis and testes were decreased, but respective relative weights were increased. There was no effect on sperm motility, density or morphology. In females, the mean cycle length of the high-dose mice was significantly increased; however there was a great variation in the length of the estrus cycle in control animals and therefore it was difficult to determine the biological significance of the findings in the treated animals.
Statistical evaluations: For body and absolute and relative organ weights, Jonckheere's test (Hollander and Wolfe, 1973) was used to determine if Williams' or Dunnett's test would be most appropriate. If the p value was less than 0.10, then Williams' test was used; greater than 0.10 Dunnett's test was used. For testing a dose-related decrease in sperm motility and for an increase in percentage abnormal sperm, Jonckheere's test for trend was used. Sperm density was tested using a Kruskal-Wallis test.
Result: Offspring toxicity F1 and F2: Not applicable
Parental data and F1: Not applicable
Test condition: The test conditions are described in National Toxicology Program (1993) for the 13-week portion of the study. In addition, an analysis was conducted in males for sperm

motility, sperm count, and sperm head morphology and in females for vaginal cytology (to evaluate the estrous cycle). In males, sperm was collected as soon as possible following termination. Sperm motility was estimated immediately. Two slides were prepared and sperm motility was estimated by determining the actual number of motile and non-motile sperm in one field of vision. Ten fields of vision were used to determine final motility. Sperm count was conducted following a 15-minute incubation period in modified Tyrode's buffer. Approximately 1 ml of sperm suspension was used to stain sperm heads with Eosin Y stain for evaluation of morphology according to the criteria of Wyrobeck and Bruce (1975). In females, vaginal smears were taken from 2 females from each dose group and controls for the last 7 days of the 13-week study. The slides were evaluated following the criteria of Allen (

Conclusion: Benzyl acetate under these test conditions did not show any effects on reproductive parameters tested.

Reliability: (1) valid without restriction
This study was conducted by the National Toxicology Program.

07-JAN-2001 (27)

Type: other:
In Vitro/in vivo: In vivo
Species: rat
Strain: other: F344 **Sex:** male
Route of administration: other: Diet
Exposure period: Premating exposure, Females: None Premating exposure, Males: None
Frequency of treatment: Daily
Duration of test: 13 weeks
Doses: 3,130, 6,250, 12,500, and 25,000 ppm
Control Group: other: Basal diet

Method: other: NTP 13-week study with sperm morphology and vaginal cytology examinations (SMVCEs)
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: In male rats, terminal body weights were decreased (specific dose levels not reported). There was no effect on reproductive organ weights, sperm motility, density or morphology. There was a great variation in the length of the estrus cycle in control animals and therefore it was difficult to determine the biological significance of any findings in the treated animals.
Statistical evaluations: For body and absolute and relative organ weights, Jonckheere's test (Hollander and Wolfe, 1973) was used to determine if Williams' or Dunnett's test would be most appropriate. If the p value was less than 0.10, then Williams' test was used; greater than 0.10 Dunnett's test was used. For testing a dose-related decrease in sperm motility and for an increase in percentage abnormal sperm,

Jonckheere's test for trend was used. Sperm density was tested using a Kruskal-Wallis test.

Result: Offspring toxicity F1 and F2: Not applicable
Parental data and F1: Not applicable

Test condition: The test conditions are described in National Toxicology Program (1993) for the 13-week portion of the study. In addition, an analysis was conducted in males for sperm motility, sperm count, and sperm head morphology and in females for vaginal cytology (to evaluate the estrous cycle). In males, sperm was collected as soon as possible following termination. Sperm motility was estimated immediately. Two slides were prepared and sperm motility was estimated by determining the actual number of motile and non-motile sperm in one field of vision. Ten fields of vision were used to determine final motility. Sperm count was conducted following a 15-minute incubation period in modified Tyrode's buffer. Approximately 1 ml of sperm suspension was used to stain sperm heads with Eosin Y stain for evaluation of morphology according to the criteria of Wyrobeck and Bruce (1975). In females, vaginal smears were taken from 2 females from each dose group and controls for the last 7 days of the 13-week study. The slides were evaluated following the criteria of Allen (

Conclusion: Benzyl acetate under these test conditions did not show any effects on reproductive parameters tested.

Reliability: (1) valid without restriction
This study was conducted by the National Toxicology Program.

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IUCLID Data Set

Existing Chemical	ID: 120-51-4
CAS No.	120-51-4
EINECS Name	benzyl benzoate
EC No.	204-402-9
Molecular Formula	C14H12O2
Producer Related Part	
Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001
Substance Related Part	
Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001
Memo:	Benzyl Derivatives
Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**
Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 21 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (2)

Value: = 21 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (14)

2.2 Boiling Point

Value: = 323 - 324 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (14)

Value: = 323.5 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (2)

2.4 Vapour Pressure

Value: = .0013 hPa at 20 degree C

Method: other (measured)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (1) valid without restriction
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (7)

Value: = .0007 hPa at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (3)

Value: = .0007 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated/Mean of Antoine & Grain method
Test condition: Calculated based on a measured boiling point of 323.5 C.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (21)

2.5 Partition Coefficient

log Pow: = 3.54 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (20)

log Pow: = 3.97 at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data

Method: Measured
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (12)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 15.39 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a log Kow = 3.97
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (22)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 18.4 hour(s)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (16)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 63 days at pH 8 and 1.7 years at pH 7
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (19)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.00E+08
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 367
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1150
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.000073
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 467
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 184
Aerosol =0.0015% Air =0.39% Fish =0.0049% Sediment =1.94%
Soil =87.1% Suspended Sediment =0.060% Water =10.5%
Test condition: Input parameters: MW, log Kow, VP, MP & calculated water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (15)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Result: other: 34.2% biodegradation after 3 days, 44.9% after day 7, 64.9% after day 10, 92.5% after day 14, 82.3% after day 18, 86.2% after day 21, 87.5% after day 24, 93% after day 28; 95% confidence limits = 76.6-109.5

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year: 1994
GLP: no data
Test substance: other TS

Result: Degradation % after time: 93% on day 28
Time required for 10% degradation: 1 day
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Innoculum: 10% by volume of secondary effluent from an unacclimatised activated sludge
The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 C. The mean percentage biodegradation was calculated from 4 vessels on

day 28
Test substance: Benzyl benzoate (99% purity)
Conclusion: Benzyl benzoate is classified as readily and ultimately biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines and is considered reliable.
12-MAR-2001 (1)

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 1.08; nonlinear - 0.999. Expert survey results: ultimate - 2.9 weeks; primary - 3.8 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Benzyl benzoate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (17)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Acute Fish Toxicity
Species: other: Zebra fish (Brachydanio rerio)/Hamilton Buchanan/West-Aquarium
Unit: **Analytical monitoring:** yes

Method: other: E.C. Council Directive 92/69/EEC C.1
Year: 1993
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: No mortalities at 3.9 mg/L 100% mortality (10/10) at 5.5 mg/L at 72 hours and 100% at 7.8 mg/L at 48 hrs. Animals at 5.5 mg/L exhibited sluggish swimming action at 48 hours. One animal swam without control. Small variations in test conditions were recorded over the 96 hours: Temp, 22.8-21.4C; [O2] = 7.8-9.5 mg/L; % O2 sat = 91-113%; pH = 7.4-8.0

Test condition: Zebra fish (10/group) acclimated for 10 weeks were exposed to 4 concentrations of amyl salicylate (3.9, 5.5, 7.8, and 11 mg/L) under semistatic conditions with daily renewal for 96 hrs. The test substance in synthetic water was treated for 60 sec at 8000 rpm with ultra turrax. Animals were exposed to 16 hours of light in synthetic fresh water. Control and test solutions were monitored for temperature, O2 concentration, % O2 saturation, and pH. The concentration of the test substance was analyzed by GC at the beginning and after each day normally during the first 2-day period. Fish were monitored for behavior and mortality daily. Body weight and length were measured at death or after sacrifice at 96 hrs.

Conclusion: For LC0, mean test concentration was 3.1 mg/L at 0 hours and 1.0 mg/L at 24 or 48 hours. For LC100, mean test concentration was 4.7mg/L at 0 hours and 1.9 mg/L at 48 hours. Based on nominal test concentrations, LC0 = 3.3 mg/L, LC100 = 4.7 mg/L, and LC50 = 3.9 mg/L. Based on measured mean concentrations, LC0 = 2.2 mg/L, LC100 = 3.3 mg/L, and LC50 = 2.7 mg/L. Based on mean final concentrations, LC0 = 0.95 mg/L, LC100 = 1.90 mg/L, and LC50 = 1.34 mg/L.

Reliability: (1) valid without restriction
Test protocol comparable to OECD semistatic test protocol.

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 96 hour LC50 = 2.8 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (18)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 48 hour LC50 = 2.7 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (18)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = .24 -

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 96 hour EC50 = 0.24 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (18)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	no data
Sex:	no data
No. of Animals:	10
Vehicle:	other:None
Method:	other: LD50 calculated by using the Draize method
Year:	1948
GLP:	no
Test substance:	as prescribed by 1.1 - 1.4
Result:	LD50 of 1.7 ml/kg bw was calculated from dosage-mortality curve. Number of deaths at each dose level: Not reported
Test condition:	No further details
Conclusion:	Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability:	(2) valid with restrictions The data were collected prior to establishment of GLP and OECD guidelines. The description of the protocol was limited and the results were tabulated.

04-MAR-2001 (5)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: other:None

Method: other: LD50 calculated by using the Draize method
Year: 1948
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 of 1.4 ml/kg bw was calculated from dosage-mortality curve.
Number of deaths at each dose level: Not reported
Test condition: No further details
Conclusion: Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability: (2) valid with restrictions
The data were collected prior to establishment of GLP and OECD guidelines. The description of the protocol was limited and the results were tabulated.
04-MAR-2001 (5)

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: other:None

Method: other: LD50 calculated by using the Draize method
Year: 1948
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 of 1.8 ml/kg bw was calculated from dosage-mortality curve.
Number of deaths at each dose level: Not reported
Test condition: No further details
Conclusion: Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability: (2) valid with restrictions
The data were collected prior to establishment of GLP and OECD guidelines. The description of the protocol was limited and the results were tabulated.
04-MAR-2001 (5)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: other:None

Method: other: LD50 calculated by using the Draize method
Year: 1948
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 of 1.0 ml/kg bw was calculated from dosage-mortality curve.
Number of deaths at each dose level: Not reported
Test condition: No further details
Conclusion: Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability: (2) valid with restrictions
The data were collected prior to establishment of GLP and OECD guidelines. The description of the protocol was limited and the results were tabulated.
04-MAR-2001 (5)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rats showed signs of central nervous system stimulation including: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis).
Result: LD50 = 2800 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 20 rats were used to determine the LD50. Not less than 3 groups of 5 rats were tested. Rats were fasted 24 to 48 hours prior to administration of test substance. Rats were observed for 2 weeks or until death.
Not reported
Conclusion: Cats, rats and rabbits appear to have a similar tolerance for orally administered benzyl benzoate.
Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to

previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.

30-JUN-2001 (8)

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rabbits showed signs of central nervous system stimulation similar to that reported in rats

[ncluding: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis)] followed by a 12- to 24-hour period of prostration prior to death.

Result: LD50 = 1680 mg/kg bw
Number of deaths at each dose level: Not reported

Test condition: A total of 12 rabbits were used to determine the LD50. Not less than 3 groups of 3 rabbits were tested. Rabbits were fasted 24 to 48 hours prior to administration of test substance. Rabbits were observed for 2 weeks or until death. Not reported

Conclusion: Cats, rats and rabbits appear to have a similar tolerance for orally administered benzyl benzoate.

Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.

30-JUN-2001 (8)

Type: LD50
Species: other: Cat
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated cats showed signs of central nervous system stimulation similar to that reported in rats and rabbits and included: excessive salivation shortly after administration, generalized tremor and muscular incoordination about 3 hours after administration, followed by progressive paralysis of the hind limbs and violent spastic seizures and then prostration prior to death (often preceded by respiratory paralysis). Death occurred within 24 hours of administration.

Result: LD50 = 2240 mg/kg bw
Number of deaths at each dose level: Not reported

Test condition: A total of 11 cats were used to determine the LD50. Cats were fasted 24 to 48 hours prior to administration of test substance. Cats were observed for 2 weeks or until death. Not reported

Conclusion: Cats, rats and rabbits appear to have a similar tolerance for orally administered benzyl benzoate.

Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was

limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.
30-JUN-2001 (8)

Type: LD50
Species: other: Dog
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: At doses 10 times that of cats, rats and rabbits, dogs showed no toxic manifestations.
Result: LD50 = >22440 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 4 dogs were used to determine the LD50. Dogs were fasted 24 to 48 hours prior to administration of test substance. Dogs were observed for 2 weeks or until death. Not reported
Conclusion: Dogs appear to show a higher tolerance (approximately 10 times) to orally administered benzyl benzoate compared to cats, rats, and rabbits.
Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.
30-JUN-2001 (8)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: other:None

Method:	other: LD50 calculated by using the Draize method
Year:	1948
GLP:	no
Test substance:	as prescribed by 1.1 - 1.4
Result:	LD50 of 4.0 ml/kg bw was calculated from dosage-mortality curve. Number of deaths at each dose level: Not reported
Test condition:	No further details
Conclusion:	Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability:	(2) valid with restrictions The data were collected prior to establishment of GLP and OECD guidelines. The description of the protocol was limited and the results were tabulated.

04-MAR-2001 (5)

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: CDSD
Route of administration: other: dermal
Exposure period: 30 days
Frequency of treatment: daily
Post exposure period: None
Doses: 188, 301, 488, 781, 1,250, or 2,000 mg neat benzyl benzoate/kg bw/d
Control Group: other: none described

Method: other: 30-day dermal toxicity study
Year: 1980
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
Result: LOAEL: 2,000 mg/kg bw/d
Toxic response/effects by dose level: During days 6 to 9, rats from the highest dose group had anogenital discharge and tremors; all the males and 1 female died. Body weight was decreased in animals of the 1,250 mg/kg bw/d dose group. No dermal reactions were reported and no biologically significant changes in hematological parameters or clinical chemistry were reported. There were no gross pathological findings; however, microscopically, minor squamous epithelial hyperplasia, degeneration of hair follicles and sebaceous glands, and subcutaneous fibrosis and hyperplasia of the thyroid gland were observed in all treatment groups.
Test condition: Groups of 3 male and 3 female Sprague-Dawley rats with topically treated with 188, 301, 488, 781, 1,250, or 2,000 mg neat benzyl benzoate/kg bw/d for a period of 30 days. No control group was used and animals were treated on different skin regions by rotation to avoid dermal reactions. Animals were observed daily, weighed weekly, and skin reactions were scored by the Draize method.
Conclusion: Without the presence of a control group, the significance of these findings is difficult to evaluate. The authors concluded that doses selected for a 90-day study should range from 188 mg/kg bw/d (no effects) to 1,000 mg/kg bw/d (effects). No effort was made to prevent oral ingestion.
Reliability: (3) invalid
GLP study

Type: Sub-acute
Species: rabbit **Sex:** male
Strain: no data
Route of administration: other: dermal
Exposure period: 90 days
Frequency of treatment: daily
Post exposure period: None
Doses: not described
Control Group: other: not reported

Method: other: 90-day dermal study (Draize, 1944)
Year: 1948
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: A 90-day LD50 of 2.0 ml/kg was calculated from dosage-mortality curve. Additional details of results were not reported.
Statistical evaluations: Not described
Result: Toxic response/effects by dose level: Atrophy of testis at 2 highest dose levels of survivors. In addition, dermatitis; possibly increased incidence of focal nephritis and encephalitis were reported.
Test condition: Benzyl benzoate was dermally applied to a total of 441 rabbits at various doses. Pathology was conducted on these animals at termination.
Conclusion: Authors concluded that benzyl benzoate acts as an 'all or nothing' toxicant, with a critical dose level of approximately 2.0 ml/kg/day based on a series of tests.
Reliability: (4) not assignable
Limited description of protocol; results tabulated.
04-MAR-2001 (4)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA1537, TA98, TA100
Concentration: 3 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Spot tests are less sensitive than quantitative experiments. Also, the results from strain TA100 are difficult to interpret because the high growth background (150-200 colonies per plate); however, spot tests provide a good "screening" method for large numbers of chemicals.

Result: Benzyl benzoate produced a negative response in this assay.
Test condition: For each experiment viable count was determined, the number of spontaneous revertants was measured, the presence of the rfa-mutation was determined by crystal violet inhibition, the presence of the plasmid pKM 101 in strains TA98 and TA100 was determined by resistance to ampicillin, and the response to positive controls N-methyl-N-nitrosoquanidin (without metabolic activation) and 2-aminoanthracene (with activation) was determined. Spectroscopic-grade ethanol was used as the solvent. The test substance was tested at 3 umol/plate in TA98, TA100, TA1535, and TA1537 with or without S9. If there was no background lawn of bacteria, the tests were redone using lower concentrations. Uncertain results prompted the conduction of the tests at 4 concentration levels (0.03, 0.3, 3 and 30 umol/plate).
Metabolic activation: with and without rat liver microsome fraction S9 from Aroclor induced rats
Conclusion: Benzyl benzoate is non-mutagenic in the Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 with or without S9.
Reliability: (1) valid without restriction
Study is published in a peer reviewed journal with adequate description and follows standard procedures.
30-MAR-2001 (6)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100
Concentration: 5, 50, 250, 500, or 5000 ug/plate
Cytotoxic Concentration: >2000 ug/plate
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1988
GLP: no
Test substance: other TS

Remark: In this assay, the concentrations of benzyl benzoate tested were bacteriocidal; however, the addition of S9 reduced toxicity.
Result: Benzyl benzoate produced no increase in reverse mutations.
Test condition: Benzyl benzoate in isopropyl alcohol was irradiated with a UV lamp or natural sunlight with or without photosensitizer (acetophenone or benzophenone). Reaction mixtures were prepared for incubation with bacteria with or without S9. Three replicates were performed.
Metabolic activation: S9 prepared from livers of male Sprague-Dawley rats
Test substance: Benzyl benzoate (reagent grade)
Conclusion: The authors concluded that the photodegradation of benzyl benzoate did not increase mutagenic activity.
Reliability: (2) valid with restrictions
Study is published in a peer reviewed journal with adequate description and follows standard procedures.

15-MAY-2001 (13)

5.6 Genetic Toxicity 'in Vivo'

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5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male
Strain: Wistar
Route of administration: other: Diet
Exposure period: 21 days
Frequency of treatment: Daily
Duration of test: 21 days
Doses: 0.04 or 1.0%
Control Group: other: No control group described
Result: NOAEL Developmental Toxicity: >1%

Year: 1980
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: There were no effects reported on the fetus relating to external, skeletal or visceral anomalies.
Result: Fetal data with dose level: No effects.
Maternal data with dose level: Not reported
NOAEL Maternal Toxicity: >1%
Test condition: Benzyl benzoate was fed to pregnant Wistar rats at concentrations of 0, 0.04, or 1.0% in the diet during gestation days 0 to 21.
Conclusion: No developmental effects reported in rats fed up to 1% benzyl benzoate during gestation days 0 to 21.
Reliability: (2) valid with restrictions
Study translated from foreign article with limited description.

16-APR-2001 (11)

5.8.3 Toxicity to Reproduction, Other Studies

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IUCLID Data Set

Existing Chemical	ID: 93-58-3
CAS No.	93-58-3
EINECS Name	methyl benzoate
EC No.	202-259-7
Molecular Formula	C8H8O2

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo:	Benzyl Derivatives
Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = -15 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (33)

Value: = -12.4 degree C

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (4)

2.2 Boiling Point

Value: = 198 - 200 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (33)

Value: = 199 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (4)

2.4 Vapour Pressure

Value: = .507 hPa at 25 degree C

Method: other (measured)
Year: 1986
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (7)

Value: = .51 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a measured boiling point of 199 C.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (41)

2.5 Partition Coefficient

log Pow: = 1.83 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (40)

log Pow: = 2.2 at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data

Method: Measured
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (26)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 2100 mg/l at 20 degree C

Method: other
Year: 1986
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Measured
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (25)

Solubility in: Water
Value: = 1344 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Calculated
Test condition: Calculated based on a log Kow = 2.12
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (42)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 12.7 day(s)

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (36)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 200 days at pH 8 and 5.5 years at pH 7
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (39)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 118000
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (35)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 5.19
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable

The data are obtained by a recognized fugacity calculation method. Data are considered

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 16.2
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (35)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.0013
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (35)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 6.59
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (35)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.59
Aerosol =8.80E-05% Air =37.2% Fish =0.00037% Sediment =0.15%
Soil =6.55% Suspended Sediment =0.0045% Water =56.1%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (35)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 1995
GLP: no data
Test substance: other TS

Result: Degradation % after time: 95.3% at 28 days
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Innoculum: 10% by volume of secondary effluent from an unacclimatised activated sludge
The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 C. The mean percentage biodegradation was calculated from 4 vessels on day 28
Test substance: Methyl benzoate (100% purity)
Conclusion: Methyl benzoate is classified as readily and ultimately biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines and is considered reliable.
16-NOV-2001 (23)

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Result: other: Biodegradation was similar between both methyl benzoate concentrations and the glucose positive control (% SOC removed: 96.2, 100, and 99.3 for 20 mg glucose/L, 10 mg methyl benzoate/L, and 20 mg methyl benzoate/L, respectively). CO

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Degradation % after time: >80% after 15 days
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
In separate 4-L Erlenmeyer flasks containing 2 L modified biochemical oxygen demand water and supplied with CO2-free air, control (no methyl benzoate), glucose (20 mg/L0 and methyl benzoate (10 and 20 mg/L) were tested. Evolved CO2 was trapped in 3 bottles containing Ba(OH)2 and connected to each flask. Test substances were added by direct weight. During the study, the flasks were shaken at ~110 ppm and temperature ranged from 23-23.6 C. Air flow through rate was regulated to provide 1-2 bubbles/second into the CO2 traps. Trapped CO2 was precipitated as BaCO3 by reacting it with 0.024 N Ba(OH)2. To determine the amount of CO2 produced, the remaining Ba(OH)2 was titrated with 0.05 N standardized HCl. At the end of the study (28 days), concentrated H2SO4 was used to acidify the flasks' contents and after overnight aeration, the concentration of soluble organic carbon (SOC) was determined by final titrations. Innoculum: Activated sludge (microbial content: 8.4 million CFU/mL) mixed liquor collected from the Downingtown Regional Water Pollution Control Center (Downingtown, PA)
Conclusion: Methyl benzoate was considered readily biodegradable.
Reliability: (1) valid without restriction
This is a GLP study using OECD guidelines.

12-AUG-2001 (3)

Type: aerobic
Inoculum: other: 10% secondary effluent from sludge from local STP
Contact time: 28 day(s)
Result: other: Mean biodegradation of 85.7% after 21 days (CI 82.4-89.2) and the authors concluded that the modification produced similar results to the standard protocol but was simpler and more readily replicated. In addition, there was no prac

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 1991
GLP: no data
Test substance: other TS

Result: Degradation % after time: 85.7% at 21 days
Time required for 10% degradation: 1 day
Total Degradation: Yes
Test condition: 10 day window criteria: Not reported
2-10 mg DOC/L at 20 C for 28 days; modification to OECD guidelines was the use of infra-red analyzers to measure CO2; 6 replicates
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: Sodium benzoate was readily biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines, is published in a peer reviewed journal and results are consistent with chemical structure.
27-APR-2001 (1)

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 0.99; nonlinear - 0.999. Expert survey results: ultimate - 3.1 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: Methyl benzoate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (37)

Type: anaerobic
Inoculum: activated sludge
Result: other: Total degradation

Method: other: Chemical oxygen demand
Year: 1976
GLP: no
Test substance: other TS

Result: Degradation % after time: 99% at <120 hours
Kinetic: 88.5 mg COD/gL
Time required for 10% degradation: <120 hours
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Contact time: Up to 120 hours
Innoculum: From activated sludge
The concentration of test material is increased during activation until it reaches 200 mg/L COD. Degradation is carried out on an initial concentration equivalent to 200 mg/L COD and continues until there is no measured decrease in COD.
Test substance: Methyl benzoate (data for structurally related substance benzoic acid)
Conclusion: Material is readily degradable.
Reliability: (2) valid with restrictions
The data were obtained prior to GLP and OECD guidelines but data are consistent with chemical structure. Some details not available but published in a peer reviewed journal.

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Acute Fish Toxicity
Species: other: Bluegill sunfish (Lepomis macrochirus)
Unit: **Analytical monitoring:** no data

Method: other: TSCA Environmental Effects Testing Guideline 797.1400
Year: 1997
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Undissolved test substance was noted at the 2 highest concentrations
No deaths occurred in controls and at concentrations up to and including 10 mg/L. Mortality was 100% at the highest concentration. Loss of equilibrium was reported in fish exposed to 20 mg/L or higher and dose-related pigmentation darkening was seen in all treatment groups. Undissolved test substance was noted at the 2 highest concentrations.
Test condition: Groups of 10 juvenille bluegill fish (average length 22 mm and weight 0.19 g) from Northeastern Biologists (Rhinebeck, NY) were exposed to solvent control (acetone), 5.0, 10, 20, 40 or 80 mg methyl benzoate/L for 96 hours in 10 L glass tanks containing 9 L of test media (methyl benzoate dissolved in acetone and diluted with reconstituted water). The reconstituted freshwater had an initial hardness of 80 mg/L (as CaCO3) and alkalinity of 11. Each test was conducted in duplicate. Oxygen content, and pH were measured at the beginning of the test and at 24, 48, 72, and 96 hours. Test temperatures ranged from 20.3 to 23.9 C. Fish were observed for signs of toxicity every 24 hours.
Conclusion: The 96 hour LC50 was 28.3 mg/L and the NOEC was 10 mg/L based on mortality and loss of equilibrium. The NOEC for color change was not determined but was expected to be <5.0 mg/L.
Reliability: (1) valid without restriction
GLP study conducted using standard methodology.
11-AUG-2001 (5)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: 96 hour LC50 = 18.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (38)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: Static freshwater toxicity test
Species: Daphnia magna (Crustacea)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Biological observations: No deaths in controls and exposures up to and including 18.8 mg/L. At higher exposures, approximately 75% of the Daphnia were dead or immobilized. Also at these higher concentrations undissolved methyl benzoate was initially reported, but was no longer observed at 24 hours.
Test condition: Nominal concentrations as mg/L: 9.38, 18.8, 37.5, 75, 150, and 300 mg/L
Ten Daphnia magna were added in pairs to 300 mL glass test chambers containing methyl benzoate dissolved in acetone and diluted with freshwater to a volume of 250 mL. The test concentrations were 9.38, 18.8, 37.5, 75, 150, and 300 mg/L. Daphnia were exposed for 48 hours. Freshwater and solvent controls were also tested. Treatments were performed in duplicate. Survival and water temperature were monitored daily; whereas pH and dissolved oxygen concentrations were measured at the beginning and end of the test.
Conclusion: The 48-hour EC50 for methyl benzoate in Daphnia was 32.1 mg/L and the no-observed-effect concentration was 18.8 mg/L.
Reliability: (1) valid without restriction
The study was conducted under GLP and followed standard methodology.
11-AUG-2001 (6)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 48 hour LC50 = 84.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (38)

Type: other: EC50 for prevention of attachment of zebra mussel
Species: other: Zebra mussel (Dreissena polymorpha)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1997
GLP: no
Test substance: other TS

Remark: Biological observations: No effect
Test condition: Fifteen zebra mussels with 5-8 mm shell length were exposed at 17 C for 48 hours to test chemical followed by a 48 hour post exposure period in untreated well water (pH = 7.9; alkalinity as CaCO3 = 107 mg/L; hardness as CaCO3 = 134 mg/L; conductivity = 281 uS/cm). Two replicates were conducted. Inhibition of attachment was assess by touching mussels with a blunt probe.
Nominal concentrations as mg/L: 0-50 (specific values not reported)
Test substance: Methyl benzoate (data for hydrolysis product, benzoic acid)
Conclusion: Benzoic acid did not prevent the attachment of zebra mussels.
Reliability: (2) valid with restrictions
Published data and reasonably well described.
27-APR-2001 (2)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = 1.4 -

Method: other: Calculated
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Conclusion: 96 hour EC50 = 1.40 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (38)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 5
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by using the Thompson method and tables of Weil
Year: 1954
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: LD50 = 3430 mg/kg bw (2.83-4.15 s.d.)
Number of deaths at each dose level: Not reported
Test condition: Groups of 5 unfasted male rats were given a single dose of test substance in a logarithmic series. The animals were observed for up to 14 days.
Reliability: (2) valid with restrictions
The data were collected prior to GLP and OECD guidelines and the description of the study was limited.
30-JUN-2001 (30)

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 24
Vehicle: other:2% starch solution
Route of admin.: other: Gavage

Method: LD50 calculated by using the Berens method
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Animals receiving the absolute lethal dose died within 2-3 hours. Initially following methyl benzoate administration, the animals became stimulated and slowly became uncoordinated, and breathing became rapid, spasms occurred and death due to cessation of respiration occurred. Animals receiving doses below the absolute lethal dose died within 14 days. There were no macroscopic lesions on the internal organs; however, the authors reported "severe expansion of the vessels in the liver" and "a plethora in the pulmonary tissue, expansion of the vessels, there were patches of vesicular emphysema with membrane rupture" in animals receiving the absolute lethal dose. The brain and adrenal gland showed no pathological changes.

Result: LD50 = 3500 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: Groups of 24 rats were intragastrically administered 1.0, 2.0, 3.0, 4.0, or 5.0 g methyl benzoate/kg bw and were observed until death (within 14 days). The results were analyzed using the Berens method. Following death, the internal organs were macroscopically and pathologically examined.
Reliability: (2) valid with restrictions
Although the study was translated from a foreign article and the data were collected prior to GLP and OECD guidelines, the description of the study indicates that the methodology used was within the current standards and therefore the data are considered reliable.
30-JUN-2001 (15)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rats showed signs of central nervous system stimulation including: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis).
Result: LD50 = 2170 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 25 rats were used to determine the LD50. Not less than 3 groups of 5 rats were tested. Rats were fasted 24 to 48 hours prior to administration of test substance. Rats were observed for 2 weeks or until death.
Not reported
Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.
30-JUN-2001 (8)

Type: LD50
Species: mouse
Strain: other: white
Sex: no data
No. of Animals: 20
Vehicle: other:2% starch solution
Route of admin.: other: Gavage

Method: LD50 calculated by using the Berens method
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Animals receiving the absolute lethal dose died within 2-3 hours. Initially following methyl benzoate administration, the animals became stimulated and slowly became uncoordinated, and breathing became rapid, spasms occurred and death due to cessation of respiration occurred. Animals receiving doses below the absolute lethal dose died within 14 days. There were no macroscopic lesions on the internal organs; however, the authors reported "severe expansion of the vessels in the liver" and "a plethora in the pulmonary tissue, expansion of the vessels, there were patches of vesicular emphysema with membrane rupture" in animals receiving the absolute lethal dose. The brain and adrenal gland showed no pathological changes.

Result: LD50 = 3000 mg/kg bw
Number of deaths at each dose level: Not reported

Test condition: Groups of 20 white mice were intragastrically administered 0.5, 1.0, 2.0, 3.0, 3.5, or 4.0 g methyl benzoate/kg bw and were observed until death (within 14 days). The results were analyzed using the Berens method. Following death, the internal organs were macroscopically and pathologically examined.

Reliability: (2) valid with restrictions
Although the study was translated from a foreign article and the data were collected prior to GLP and OECD guidelines, the description of the study indicates that the methodology used was within the current standards and therefore the data are considered reliable.

30-JUN-2001 (15)

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated
Year: 1945
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Overall intoxicated rabbits showed signs of central nervous system stimulation similar to that reported in rats [ncluding: piloerection, muscular incoordination, progressive paralysis of the hind limbs and violent spastic convulsions, dyspnea and death (often preceded by respiratory paralysis)] followed by a 12- to 24-hour period of prostration prior to death.
Result: LD50 = 2170 mg/kg bw
Number of deaths at each dose level: Not reported
Test condition: A total of 12 rabbits were used to determine the LD50. Not less than 3 groups of 3 rabbits were tested. Rabbits were fasted 24 to 48 hours prior to administration of test substance. Rabbits were observed for 2 weeks or until death.
Not reported
Reliability: (2) valid with restrictions
This study was conducted prior to the establishment of GLP and OECD guidelines. The description of the study was limited; however, the authors did report similar findings to previous reports and the study shows good structure-activity relationships. Therefore the data are considered reliable.
30-JUN-2001 (8)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: no data
No. of Animals: 6
Vehicle: other:2% starch solution
Route of admin.: other: Gavage

Method: LD50 calculated by using the Berens method
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Animals receiving the absolute lethal dose died within 2-3 hours. Initially following methyl benzoate administration, the animals became stimulated and slowly became uncoordinated, and breathing became rapid, spasms occurred and death due to cessation of respiration occurred. Animals receiving doses below the absolute lethal dose died within

14 days. There were no macroscopic lesions on the internal organs; however, the authors reported "severe expansion of the vessels in the liver" and "a plethora in the pulmonary tissue, expansion of the vessels, there were patches of vesicular emphysema with membrane rupture" in animals receiving the absolute lethal dose. The brain and adrenal gland showed no pathological changes.

Result: LD50 = 4100 mg/kg bw
Number of deaths at each dose level: Not reported

Test condition: Groups of 6 guinea pigs were intragastrically administered 2.0, 3.0, 4.0, 5.0, or 6.0 g methyl benzoate/kg bw and were observed until death (within 14 days). The results were analyzed using the Berens method. Following death, the internal organs were macroscopically and pathologically examined.

Reliability: (2) valid with restrictions
Although the study was translated from a foreign article and the data were collected prior to GLP and OECD guidelines, the description of the study indicates that the methodology used was within the current standards and therefore the data are considered reliable.

30-JUN-2001 (15)

5.1.2 Acute Inhalation Toxicity

-

Type: LD50
Species: rat/Carworth-Wistar
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: Inhalation
Method: LD50 calculated using the Thompson method and tables of Weil
Year: 1954
GLP: no
Test substance: as prescribed by 1.1 - 1.4
Remark: No deaths observed at any exposure level
Result: LD50 = >1000 ppm
Test condition: Groups of 6 adult rats were exposed to an atmospheres saturated with methyl benzoate for 8 hours. Nominal concentrations were increased by 2 logarithmic orders. No dealths for up to 14 days post-treatment.
Reliability: (2) valid with restrictions
The data were collected prior to GLP and OECD guidelines and the description of the study was limited.

30-JUN-2001 (30)

5.1.3 Acute Dermal Toxicity

Type:	LD50
Species:	rabbit
Strain:	New Zealand white
Sex:	male/female
No. of Animals:	5
Vehicle:	other:None
Method:	other: LD50 calculated
Year:	1995
GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4
Remark:	Within the first 3 days of application, fecal staining and dark material around the facial area were reported, but considered attributable to collars placed on animals. At the application site, irritation was noted. Four male and 3 female rabbits showed decreased body weight on the first day and 1 female had reduced body weight during day 4-7. No gross findings were reported at necropsy, but cysts on the oviduct were reported in 3 females. These cysts were considered to be normal for this strain of rabbit.
Result:	LD50 = >2,000 mg/kg bw Number of deaths at each dose level: No deaths were reported.
Test condition:	Animals were given a single application of 2,000 mg methyl benzoate/kg bw and observed for 14 days. Body weights were taken on days 1, 2, 4, 7, and 14.
Reliability:	(1) valid without restriction The study was conducted under GLP.

16-NOV-2001 (18)

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: F344/N
Route of administration: oral feed
Exposure period: 18 to 24 months
Frequency of treatment: daily
Doses: 1 or 2% sodium benzoate in the diet (approximately 370 or 735 mg/kg bw/d for males and 445 or 880 mg/kg bw/d for females, respectively) Actual dose: 735 to 880 mg/kg bw/d
Control Group: other: basal diet only

Method: other: Carcinogenicity assay
Year: 1980
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. T-test
Result: Toxic response/effects by dose level: Mortality averaged 14.5% in all rat groups within the first 16 months of the study. All dead rats, with the exception of 1 female control rat, had pneumonia with abscess. After 16 months, 100 rats in total from all groups died of hemorrhagic pneumonia with edema. There were no reported differences in mortality, growth or food intake among treated and control rats. There also was no significant difference in benign (chromophobe adenoma of the pituitary, endometrial polyp, fibroadenoma of the mammary gland, and interstitial cell

tumor of the testis) and malignant tumors (leukemia, malignant lymphoma, epidermoid carcinoma of the maxilla) in treated rats compared to controls.

Test condition: Groups of 50 male and 52 female Fischer 344 rats were fed 1 or 2% sodium benzoate in the diet (approximately 370 or 735 mg/kg bw/d for males and 445 or 880 mg/kg bw/d for females, respectively) for a period of 18 to 24 months. Control rats consisting of 25 males and 43 females received basal diet alone. Rats were group housed (5 rats/cage) and monitored for clinical signs, mortality, growth, food intake, and behavior. Body weight and food intake were recorded. An interim necropsy was conducted in the middle of the study on several rats randomly selected from each group. Necropsies also were conducted at study termination. During necropsy various organ tissues were prepared for microscopic examination (specifics not reported).

Test substance: Methyl benzoate (data for structurally related substance, sodium benzoate)

Conclusion: It was concluded that sodium benzoate at up to 2% in the diet of Fischer 344 rats was not carcinogenic.

Reliability: (3) invalid
Hemorrhagic pneumonia with edema affected a significant number of the rats from all groups.

28-MAR-2001 (31)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: white (strain not specified)
Route of administration: other: not specified
Exposure period: 45 days
Frequency of treatment: daily
Doses: 111 or 500 mg/kg bw/d
Control Group: other: yes, but not described

Method: other: 1.5-month toxicity study
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
The results were difficult to interpret due to the poor translation of the article. The exact administration route and schedule were not clearly indicated and it is possible that additional groups were tested.

Result: LOAEL: 111 mg/kg bw/d
Toxic response/effects by dose level: At 111 mg/kg bw/d, there was a statistically significant increase in blood erythrocytes ($p<0.05$) and reticulocytes significantly increased. At 500 mg/kg bw/d there was no change in "red blood", but a significant increase in "white blood" ($p<0.01$) and in the number of reticulocytes. There was a significant increase in prothrombin time ($p<0.01$) at both doses and a tendency for a decreased phagocytic index (authors reported this to occur in mice, but the reviewer assumes this is a

typographical error and "rats" was intended) at 500 mg/kg bw/d. Whole blood cholinesterase activity was significantly decreased at the high dose. No histological findings were reported, although it was reported that the level of ascorbic acid in the adrenal glands was decreased in rats given 500 mg methyl benzoate/kg bw/d.

Test condition: The study was translated from a Russian study and some of the methodology was difficult to interpret. Based on the previous acute portion of the study, it appears that methyl benzoate was administered by gavage; however in a discussion of some results, the authors indicate that a "preparation" was given by intraperitoneal injection. General condition and some hematology were evaluated once every 10-11 days and at the end of the study a necropsy was performed.

Reliability: (4) not assignable
The study was translated from a foreign article and the data were collected prior to GLP and OECD guidelines and although the acute portion of this study received a code 2, the description of the subacute portion of the study is not well documented and difficult to interpret. Therefore the data are not considered reliable.

10-JUL-2001 (15)

Type: Sub-acute
Species: rat **Sex:** male
Strain: other: white (strain not specified) and grey rat
Route of administration: other: not specified
Exposure period: 6 months
Frequency of treatment: daily
Doses: 0.005 or 0.05 mg/kg bw/d
Control Group: other: yes, but not described

Method: other: 6-month toxicity study
Year: 1970
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Statistical evaluations: Not described
The results were difficult to interpret due to the poor translation of the article. The exact administration route and schedule were not clearly indicated.

Result: LOAEL: 0.05 mg/kg bw/d
NOAEL: 0.005 mg/kg bw/d
Toxic response/effects by dose level: The general condition of the animals in both dose groups did not differ from controls. At the high dose, there was decrease in the number of reticulocytes ($p<0.01$). There was no difference from controls in prothrombin time or phagocytic activity at either dose. In the grey rats, at the high dose, the latent period for response to "bell" or "light" stimulus was increased. Also, there was an increase in the number of sulfhydryl groups in cerebral tissue of high-dose grey rats. At necropsy, congestion and swelling of the hepatic central veins and capillaries was reported in high-dose rats. There

were no histological findings in the low-dose animals.

Test condition: The study was translated from a Russian study and some of the methodology was difficult to interpret. Based on the previous acute portion of the study, it appears that methyl benzoate was administered by gavage; however in a discussion of some results, the authors indicate that a "preparation" was given by intraperitoneal injection. General condition and some hematology were evaluated once every 10-11 days and at the end of the study a necropsy was performed. In addition, the grey rats were studied for conditional reflexes using a food motive method and the white rats were studied for biochemical parameters.

Conclusion: The authors reported that methyl benzoate administered at 0.005 mg/kg bw/d to rats did not produce adverse effects.

Reliability: (4) not assignable
The study was translated from a foreign article and the data were collected prior to GLP and OECD guidelines and although the acute portion of this study received a code 2, the description of the chronic portion of the study is not well documented and difficult to interpret. Therefore the data are not considered reliable.

10-JUL-2001 (15)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Wistar
Route of administration: other: oral
Exposure period: 18 months
Frequency of treatment: daily
Post exposure period: None
Doses: 40 mg benzoic acid/kg bw/d or 40 mg benzoic acid/kg bw/d plus 80 mg sodium bisulphite/kg bw/d
Control Group: other: basal diet only

Method: other: 18-month oral toxicity study
Year: 1970
GLP: no
Test substance: other TS

Remark: Statistical evaluations: No
Result: NOAEL: 40 mg/kg bw/d
Toxic response/effects by dose level: The results were not clearly reported; however, it appeared that survival was decreased in rats fed the benzoic acid/sodium bisulphite combination and that the benzoic acid/sodium bisulphite combination-fed rats showed more of an effect in the stress tests, had increased erythrocyte sedimentation rates, and a decreased level of blood ketones compared to controls. No effects on blood alkalinity, C-reactive protein levels and blood morphology were reported in the treated rats. Apparently, small groups of benzoic acid-fed rats and benzoic acid/sodium bisulphite combination-fed rats were administered a lethal dose of sodium benzoate, to which the benzoic acid-fed rats appeared to have gained tolerance

(25,100, and 100% mortality in benzoic acid-fed rats, benzoic acid/sodium bisulphite combination-fed rats, and controls, respectively).

Test condition: In an 18-month study, groups of 10 male and 10 female Wistar rats were fed 40 mg benzoic acid/kg bw/d in a paste prior to normal feeding. Similarly, groups of 50 male and 50 female Wistar rats were fed 40 mg benzoic acid/kg bw/d in conjunction with 80 mg sodium bisulphite/kg bw/d. Control animals received basal diet only. Parameters measured were food and water consumption, and body weight gain. Also, possible stress factors (low temperature tolerance) were recorded. Titre of serum complement, phagocytic activity of leucocytes, serum-ceruloplasmin level, blood alkalinity, blood ketones, blood morphology, erythrocyte sedimentation rate and C-reactive protein in serum were estimated.

Test substance: Methyl benzoate (data for structurally related substance, benzoic acid)

Conclusion: A combination of benzoic acid and sodium bisulphite apparently decreased survival in rats; however, no statistical analysis was conducted and only 1 dose level was tested.

Reliability: (3) invalid
Methodology was unconventional and there was only use of 1 dose. No statistical analyses were conducted and results were not clearly described.

28-MAR-2001 (28)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: other: cross-bred white
Route of administration: gavage
Exposure period: 3 months
Frequency of treatment: daily
Post exposure period: None
Doses: 80 mg benzoic acid/kg bw/d or 80 mg benzoic acid/kg bw/d plus 160 mg sodium bisulphite/kg bw/d
Control Group: other: basal diet only

Method: other: 3-month gavage study
Year: 1970
GLP: no
Test substance: other TS

Remark: Statistical evaluations: No
Result: LOAEL: 80 mg/kg bw/d
Toxic response/effects by dose level: Survival was decreased at 2.5 months in animals given the benzoic acid/sodium bisulphite combination. Percent survival was 60, 68, and 30% for control mice, benzoic acid-fed mice, and benzoic acid/sodium bisulphite combination-fed mice, respectively. Treated animals did not gain weight to the same extent as controls (no statistical analysis) and did not appear to be associated with feed intake. Under 90% feed restriction conditions, treated mice showed greater

mortality and weight loss than controls. Treated mice also appeared to be more sensitive to carbon tetrachloride poisoning. In the co-carcinogenicity tests, tumor growth appeared to be increased in treated animals compared to controls (data not shown by the study authors).

Test condition: Groups of 50 male and 50 female cross-bred white mice were administered 80 mg benzoic acid/kg bw/d by gavage for 3 months. Similarly, groups of 50 male and 50 female cross-bred white mice were administered 80 mg benzoic acid/kg bw/d in conjunction with 160 mg sodium bisulphite/kg bw/d. Observations consisted of general condition, behaviour, and survival. Food consumption and body weight gain were recorded daily. Additionally, mice were tested to determine the possible effects of hunger, physical stress, and poisoning with carbon tetrachloride (single dose of 0.1 ml/mouse). A co-carcinogenicity test was conducted with Ehrlich ascites carcinoma. A control group was used but not described.

Test substance: Methyl benzoate (data for structurally related substance, benzoic acid)

Conclusion: It appeared that benzoic acid administered at 80 mg/kg bw/d produced a reduction in body weight gain; however, no statistical analyses were conducted. In addition, benzoic acid in combination with sodium bisulphite appeared to decrease survival.

Reliability: (3) invalid
Methodology was unconventional and there was only use of 1 dose. No statistical analyses were conducted and results were not clearly described.

21-MAR-2001 (29)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: no data
Route of administration: other: oral
Exposure period: 17 months
Frequency of treatment: daily
Post exposure period: Not reported
Doses: 40 mg/kg bw/d
Control Group: other: not reported

Method: other: 17-month oral toxicity study
Year: 1970
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: Survival (%) at 2.5 months was greater for the benzoic acid group (68%) than that for the control group of males (60%) or female rats (62%).
Test condition: Groups of 25 male and 25 female mice were orally administered 40 mg benzoic acid/kg bw/d for 17 months.
Test substance: Methyl benzoate (data for structurally related substance

benzoic acid)
Reliability: (4) not assignable
Very limited description of study and results.
12-MAR-2001 (27)

Type: Sub-acute
Species: mouse **Sex:** male
Strain: other: albino Swiss
Route of administration: other: drinking water
Exposure period: life span (up to approximately 112 weeks)
Frequency of treatment: daily
Post exposure period: None
Doses: 2% in the drinking water (approximately 4,000 mg/kg bw/d)
Control Group: other: untreated drinking water

Method: other: Carcinogenicity assay
Year: 1984
GLP: no
Test substance: other TS

Remark: Statistical evaluations: No
Result: Toxic response/effects by dose level: Sodium benzoate administration had no effect on survival or on tumor incidence.
Test condition: Groups of 50 male and 50 female 5-week-old albino Swiss mice were administered sodium benzoate at a concentration of 2% in the drinking water (approximately 4,000 mg/kg bw/d) for their life span (up to approximately 112 weeks). Control groups consisted of 100 untreated mice per sex. Mice were examined clinically, weighed and gross pathological changes were recorded. Complete necropsies were conducted on all mice and organs were examined macroscopically and selected tissues (liver, spleen, kidney, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, and lung) were histopathologically examined.
Test substance: Methyl benzoate (data for structurally related substance, sodium benzoate)
Conclusion: Sodium benzoate at a lifetime exposure of 2% in the drinking water of mice showed no evidence of carcinogenicity.
Reliability: (2) valid with restrictions
The protocol and results were reported in a short communication and statistical analyses were not reported.
28-MAR-2001 (34)

5.5 Genetic Toxicity 'in Vitro'

Type: other: reverse mutation test
System of testing: bacterial Sd-4-73 E. coli
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Paper disk method (Iyer and Szybalski, 1958) Not reported
Year: 1958
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Methyl benzoate produced no increase in the frequency of reversion from streptomycin dependence to independence in Sd-4-73 E. coli.
Test condition: E. coli was cultured overnight at 36 C in an aerated nutrient broth containing 20 ug/ml streptomycin. Plates were prepared and methyl benzoate was added by applying to a paper disk, which was then placed on the agar. Relative mutagenicity, defined as “an approximate ratio of the number of colonies on the plate containing the mutagen to the number of colonies on the control plate”, was calculated. “Potent” mutagens had relative mutagenicities of >3 and “weak and doubtful” mutagens had relative mutagenicities between 1.5 and 3.
Metabolic activation: no
Conclusion: Methyl benzoate was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Study was conducted prior to establishment of GLP guidelines.
04-APR-2001 (32)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA1535, TA1537, TA97, TA98
Concentration: 0, 10, 33, 100, 333, 666, 1000, 1666, 3333, or 6666 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1992
GLP: no
Test substance: other TS

Result: Methyl benzoate showed no mutagenic activity in any of the strains tested with or without S9.
Test condition: Metabolic activation: S9 fractions of Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamster livers
Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine

(TA98 and 1538), mitomycin C (TA102), methyl methanesulfonate (TA104) and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains without S9. 2-Aminoanthracene was used with all strains incubated with S9 and either sterigmatocystin or 2-aminoanthracene was used for TA102.DMSO was used as the solvent control. Nine concentrations of the methyl benzoate (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 C for 48 hours. The number of revertants was machine counted. If a chemical was not active (with or without metabolic activation) in all Salmonella strains tested, it was considered non mutagenic.

Test substance: Methyl benzoate (99% purity)
Conclusion: Methyl benzoate was non mutagenic.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.

04-APR-2001 (45)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100, TA1535, and TA1537
Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1975
GLP: no
Test substance: other TS

Result: Benzoic acid produced negative results.
Test condition: Metabolic activation: S9 from liver extract of Aroclor 1254-induced rats
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid, 99.6% purity)
Conclusion: Benzoic acid was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Data were collected prior to GLP and OECD guidelines but by a method considered standard and under the direction fo a recognized research institute. Therefore the data are considered reliable.

04-JUL-2001 (17)

Type: Ames test
System of testing: bacterial Salmonella typhimuriumTA98 and TA1535
Concentration: 0, 0.1, 0.5, 1.0, 2.5, or 5.0 umol/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay Mean of 3 values with extremes never removed from the means by >5-10%.
Year: 1983
GLP: no
Test substance: other TS

Result: There was no detectable mutagenic activity.
Test condition: Metabolic activation: not reported
The test substance was tested at 5 concentrations with 3 plates per concentration. The positive control for TA1535 was 2 ug sodium azide and for TA98 was 3 ug 2-nitrofluorene.
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid)
Conclusion: Benzoic acid was non-mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
03-JUL-2001 (44)

Type: Sister chromatid exchange assay
System of testing: non bacterial Human lymphocytes
Concentration: 0-2.0 mM
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: negative

Method: other: Sister chromatid exchange (Jansson et al. 1986) The data were analyzed using linear regression by least squares and significance was tested at $p<0.05$, 0.01, and 0.001.
Year: 1988
GLP: no
Test substance: other TS

Result: No statistically significant increase in sister chromatid exchanges as compared to the vehicle control. The regression coefficient was -0.5 SCE/cell/mM.
Test condition: DMSO and ethanol were used as solvents and negative controls. The positive control used was styrene-7,8-oxide. After an exposure of 88 hours, the lymphocytes were treated with colchicine (50 ng/ml for 2 hours) and hypotonic KCl (0.075 M for 5-10 minutes). For each concentration tested (not specified), 25 metaphases from one culture were analysed.
Metabolic activation: Phytohemagglutinin-stimulated
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid, 99.6% purity)
Conclusion: Benzoic acid did not induce sister chromatid exchanges in this assay.

Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal.
08-JUL-2001 (12)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: other TS

Result: No mutagenic activity was reported
Test condition: Metabolic activation: none
The test was not conducted in duplicate and was part of a larger study examining the mutagenicity of aqueous chlorination of organic compounds.
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid)
Conclusion: Benzoic acid was non-mutagenic in this assay.
Reliability: (3) invalid
The assay was not conducted in accordance with current standards (lack of duplicates) and was not well described.
03-JUL-2001 (24)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA97, TA98, TA100, TA1535, and TA1537
Metabolic activation: with
Result: negative

Method: other: Ames assay (modified) (Haworth et al., 1983)
Year: 1988
GLP: yes
Test substance: other TS

Result: No mutagenic activity was reported.
Test condition: Metabolic activation: S9 fractions of Aroclor 1254-induced male SD rat and male Syrian hamster livers
Toxicity was tested in a preliminary test. Benzoic acid was incubated with Salmonella without shaking at 37 C for 20 min. The test solution was plated onto petri dishes containing Vogel-Bonner medium and revertant colonies were machine-counted following a 2-day incubation period. Positive controls used were sodium azide, 9-aminoacridine, 4-nitro-o-phenylenediamine, and 2-aminoanthracene. Benzoic acid was tested 2X in the same laboratory.
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid, 99% purity)
Conclusion: Benzoic acid was non-mutagenic in this assay.

Reliability: (1) valid without restriction
NTP study
03-JUL-2001 (46)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA1535, TA100, TA1537, TA1538, and TA98
Concentration: not reported
Metabolic activation: with
Result: negative

Method: other: Ames assay
Year: 1976
GLP: no
Test substance: other TS

Result: No mutagenic activity was reported.
Test condition: Benzoic acid was applied as a solid in 100 ug amounts to the center of agar plates seeded with tester strains.
Metabolic activation: not reported
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid)
Conclusion: Benzoic acid was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the results were published in a peer-reviewed journal. Therefore the data are considered reliable.
03-JUL-2001 (19)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 10 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: other TS

Result: Benzoic acid produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid,

99.6% purity)
Conclusion: Benzoic acid was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-JUL-2001 (11)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100 and TA98
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: other TS

Result: Sodium benzoate produced negative results.
Test condition: Metabolic activation: none
Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate)
Conclusion: Sodium benzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (14)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 1.5 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: other TS

Result: Benzoic acid produced equivocal results with 1.0% polyploid cells and 8% structural chromosomal aberrations.
Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells

and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none

Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid, 99.6% purity)

Conclusion: Benzoic acid produced equivocal results in this assay.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-JUL-2001 (9)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis (strain not specified)
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1980
GLP: no
Test substance: other TS

Result: Sodium benzoate produced positive results without metabolic activation.

Test condition: Metabolic activation: none

Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate)

Conclusion: Sodium benzoate produced positive results in this assay without metabolic activation but based on weight of evidence, the authors did not consider sodium benzoate to have mutagenic or carcinogenic potential.

Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.

03-JUL-2001 (14)

Type: Chromosomal aberration test
System of testing: non bacterial Hamster lung fibroblast cells
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations
Year: 1980
GLP: no
Test substance: other TS

Result: Sodium benzoate produced positive results without metabolic activation.
Test condition: Metabolic activation: none
Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate)
Conclusion: Sodium benzoate produced positive results in this assay without metabolic activation but based on weight of evidence, the authors did not consider sodium benzoate to have mutagenic or carcinogenic potential.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (14)

Type: Sister chromatid exchange assay
System of testing: non bacterial Hamster lung fibroblast cells
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Sister chromatid exchange
Year: 1980
GLP: no
Test substance: other TS

Result: Sodium benzoate produced negative results.
Test condition: Metabolic activation: none
Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate)
Conclusion: Sodium benzoate was non-clastogenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (13)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 3 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: other TS

Result: Sodium benzoate produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.
Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate, 99% purity)
Conclusion: Sodium benzoate was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-JUL-2001 (10)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98 and TA100
Concentration: 0.1 mg/disc
Metabolic activation: with
Result: positive

Method: other: Ames assay (modified)
Year: 1992
GLP: no
Test substance: other TS

Result: Sodium 2-hydroxybenzoate produced negative results in both tester strains.
Test condition: Metabolic activation: S9 from livers of PCB-treated male SD rats, male ddY mice, male golden hamsters, or male Hartley guinea pigs.
Sodium salicylate was preincubated with the tester strain at 37 C for 30 min in a shaking water bath. After plating onto petri dishes containing Vogel-Bonner E medium, the dishes were incubated for 48 hours and revertants were counted. Induction of more than 2X the number of spontaneously

occurring revertants was considered positive. Positive controls used were furyl furamide, benzopyrene, and 4-nitroquinoline-1-oxide.

Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate)

Conclusion: Sodium 2-hydroxybenzoate was non-mutagenic in this assay.

Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (16)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98 and TA100
Concentration: 0.1 mg/disc
Metabolic activation: with
Result: positive

Method: other: Ames assay (modified)
Year: 1992
GLP: no
Test substance: other TS

Result: Benzoic acid produced negative results in both tester strains.

Test condition: Benzoic acid was preincubated with the tester strain at 37 C for 30 min in a shaking water bath. After plating onto petri dishes containing Vogel-Bonner E medium, the dishes were incubated for 48 hours and revertants were counted. Induction of more than 2X the number of spontaneously occurring revertants was considered positive. Positive controls used were furyl furamide, benzopyrene, and 4-nitroquinoline-1-oxide.
Metabolic activation: S9 from livers of PCB-treated male SD rats, male ddY mice, male golden hamsters, or male Hartley guinea pigs.

Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid)

Conclusion: Benzoic acid was non-mutagenic in this assay.

Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (16)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 5 mg/disc
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay (Kada and Sadaie, 1972)
Year: 1992
GLP: no
Test substance: other TS

Result: Benzoic acid produced positive results with a zone difference of 2.9 mm.
Test condition: After plating on petri dishes, plates were held at 4 C for 24 hours followed by a 24-hour incubation at 37 C. Afterwards, the length of the inhibition zone was measured. An inhibition zone of >2mm difference between growth inhibition zones for Rec+ and Rec- was considered to be indicative of DNA damage.
Test substance: Methyl benzoate (data on hydrolysis product, benzoic acid)
Conclusion: The authors concluded that benzoic acid has weak DNA damaging potential.
Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.
03-JUL-2001 (16)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 2.0 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: other TS

Result: Sodium benzoate produced positive results with 1.0% polyploid cells and 38% structural chromosomal aberrations.
Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated

cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none

Test substance: Methyl benzoate (data on hydrolysis product, sodium benzoate, 99% purity)

Conclusion: Sodium benzoate was clastogenic in this assay.

Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.

04-JUL-2001 (9)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Clastogenetic study
Species: other: Rat bone marrow cells **Sex:** no data
Route of admin.: unspecified
Exposure period: Not reported
Doses: Not reported
Result: negative

Method: other: Chromosomal aberrations
Year: 1980
GLP: no
Test substance: other TS

Remark: Sodium benzoate produced negative results.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: Sodium benzoate was non-clastogenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.

03-JUL-2001 (14)

Type: other: Mutation
Species: other: Silk worm **Sex:** no data
Route of admin.: unspecified
Exposure period: Not reported
Doses: Not reported
Result: negative

Year: 1980
GLP: no
Test substance: other TS

Remark: Sodium benzoate produced negative results.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: Sodium benzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (14)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male
Strain: Wistar
Route of administration: other: Diet
Exposure period: Gestation
Frequency of treatment: Daily
Duration of test: up to 11 weeks
Doses: 0, 1, 2, 4, or 8% sodium benzoate in the diet (approximately 0, 667, 1333, 1600, or 710 mg/kg bw/d)
Control Group: other: 0% in the diet for gestation
Result: NOAEL Developmental Toxicity: 1333 mg/kg bw/d LOAEL Developmental Toxicity: 1600 mg/kg bw/d

Year: 1978
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: Fetal data with dose level: At the 2 highest concentrations, there was an increase in the number of dead fetuses and the number of resorbed embryos, body weight of viable fetuses was reduced, mild systemic edema was seen, an increased number of fetal abnormalities (microphthalmia, anophthalmia, hydrocephalus, pyelectasis, renal hydroplasia, cerebral hypoplasia) was reported (0/1, 1/159, 1/183, 12/151, and 11/117 fetuses with abnormalities out of fetuses examined for control, 1, 2, 4, and 8% groups, respectively). The skeletal anomalies most frequently observed were “dysplasia of lumbar ribs” (incidence: 23, 23, 31, 43, and 16% for

control, 1, 2, 4, and 8% groups, respectively) and “varied sternebrae” (incidence: 36, 37, 36, 97, and 100% for control, 1, 2, 4, and 8% groups, respectively). In rats allowed to naturally come to term and deliver their offspring, number of pups born decreased, number of perinatal deaths increased (to 100%), lactation rate decreased (to 0%), and survival rate decreased (to 0%) at the 2 highest doses. The pups terminated at 8 week
LOAEL Maternal Toxicity: 1600 mg/kg bw/d
Maternal data with dose level: Maternal body weight gain and food consumption were significantly decreased at the 2 highest concentrations with rats losing weight at the highest dose. There were 2 deaths at 4% and 3 deaths at 8%.
NOAEL Maternal Toxicity: 1333 mg/kg bw/d

Test condition: Groups of 27-30 pregnant Wistar rats were fed 0, 1, 2, 4, or 8% sodium benzoate in the diet (approximately 0, 667, 1333, 1600, or 710 mg/kg bw/d based on data provided in the study) during gestation. On gestation day 20, 22-25 rats from each group were killed and examined for the number of viable fetuses, number of dead fetuses, number of absorbed embryos, number of placenta, number of implantation sites, fetal weight, placental weight, and ovary weight. Approximately 75% of the fetuses were prepared by alizarin Red S staining for assessing skeletal abnormalities. The remaining fetuses underwent fixation with Bouin’s fluid for examination of abnormalities in the head and thoracic and abdominal regions. Five rats from each group were allowed to terminate their pregnancy naturally and the number of pups, survival, appearance, and body weight were recorded. After 3 weeks, the pups were weaned and grossly examined. Half of the pups were killed and treated as previously described for the fetuses. The remaining pups were maintained for another 5 weeks and w

Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)

Conclusion: Effects on the fetus occurred only at maternally toxic dietary concentrations of 4% sodium benzoate or higher.

Reliability: (2) valid with restrictions
The study was reported in Japanese with an English summary.

28-MAR-2001 (21)

Species: other: Chicken/Single-Comb White Leghorn **Sex:** male
Strain: other: Single-Comb White Leghorn
Route of administration: other: Egg
Exposure period: Single injection at 0 hour and 96 hour of incubation
Frequency of treatment: 2 single injections
Duration of test: day 0 of incubation to hatching
Doses: minimum of 5 dose levels (not specified); highest dose tested was mg/egg
Control Group: other: Solvent control (water) at a volume of 100 uL or less

Method: other (calculated): Air cell injection of chicken egg methodology of McLaughlin et al., 1964 and Verrett et al., 1964., Teratology-calculated LD50
Year: 1980
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results analyzed used X2 test (Snedecor and Cochran, 1967)
Result: Fetal data with dose level: No teratogenic effects reported. Maternal data with dose level: Not applicable
Test condition: Fresh fertile eggs were injected through the air cell with up to 5 mg sodium benzoate/egg in water solution of a maximum volume of 100 uL. Control were injected with water only. The eggs were injected twice during incubation: 0 hour and 96 hour. The eggs were candled daily and surviving embryos were allowed to hatch. The chicks underwent gross examination and those with skeletal defects were x-rayed or stained with Alizarin Red S. Percent mortality was used to calculate the LD50.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: The LD50 was reported to be 4.74 mg/egg.
Reliability: (1) valid without restriction
The study was conducted by the FDA and is considered reliable.

Species: rat **Sex:** male
Strain: Wistar
Route of administration: other: Oral intubation
Exposure period: Gestation days 6-15
Frequency of treatment: Daily
Duration of test: 20 days
Doses: 0, 1.75, 8.0, 38, or 175 mg sodium benzoate/kg bw/d
Control Group: other: Sham-treated or 250 mg aspirin/kg bw/d (positive control)
Result: NOAEL Developmental Toxicity: >175 mg/kg bw/d

Year: 1972
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: Fetal data with dose level: No soft tissue abnormalities were reported in any of the test groups with the exception of subcutaneous edema in one pup of the 38 mg/kg bw/d group and exencephaly, spina bifida and enterohepatocele in some pups from the positive control group. Various skeletal abnormalities (i.e., incomplete ossification of the sternebrae, bipartite and missing sternebrae, wavy ribs, incomplete ossification of the ribs and vertebrae, incomplete closure of the skull, and missing or reduced hyoid) were reported in all test groups including the sham-treated control, but there were no differences between sodium benzoate-treated rats and sham-treated controls. Maternal data with dose level: No significant differences between control and sodium benzoate-treated rats were reported.
NOAEL Maternal Toxicity: >175 mg/kg bw/d
Test condition: Groups of 23-24 pregnant Wistar-derived albino rats were administered 0, 1.75, 8.0, 38, or 175 mg sodium benzoate/kg bw/d or 250 mg aspirin/kg bw/d (positive control) by oral intubation as a water solution during gestation days 6 to 15 inclusive. Appearance, behavior, and food consumption were monitored daily. Body weights were recorded on gestation days 0, 6, 11, 15, and 20. On gestation day 20, dams underwent Caesarean section. The number of resorption and implantation sites, and live and dead fetuses, and live fetal body weight were recorded. Fetuses were examined for skeletal and soft tissue abnormalities by gross examination and alizarin red S dye staining.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: The author concluded “The administration of up to 175 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation of on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.”
Reliability: (1) valid without restriction

Although description of protocol was limited and the results were tabulated, the study was conducted for the FDA and was considered reliable.

16-NOV-2001 (20)

Species: mouse **Sex:** male
Strain: other: albino CD-1 outbred
Route of administration: other: Oral intubation
Exposure period: Gestation days 6-15
Frequency of treatment: Daily
Duration of test: 17 days
Doses: 0, 1.75, 8.0, 38, or 175 mg sodium benzoate/kg bw/d
Control Group: other: Sham-treated or 150 mg aspirin/kg bw/d (positive control) in water solution
Result: NOAEL Developmental Toxicity: >175 mg/kg bw/d

Year: 1972
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: Fetal data with dose level: No soft tissue abnormalities were reported in any of the test groups. Various skeletal abnormalities (i.e., incomplete ossification of the sternebrae, bipartite and missing sternebrae, wavy ribs, incomplete ossification of the vertebrae and extremities, and missing or reduced hyoid) were reported in all test groups including controls, but there were no differences between sodium benzoate-treated mice and controls.
Maternal data with dose level: No significant differences between control and sodium benzoate-treated mice were reported.
NOAEL Maternal Toxicity: >175 mg/kg bw/d
Test condition: Groups of 20-21 pregnant albino CD-1 outbred mice were administered 0, 1.75, 8.0, 38, or 175 mg sodium benzoate/kg bw/d or 150 mg aspirin/kg bw/d (positive control) by oral intubation as a water solution during gestation days 6 to 15 inclusive. Appearance, behavior, and food consumption were monitored daily. Body weights were recorded on gestation days 0, 6, 11, 15, and 17. On gestation day 17, dams underwent Caesarean section. The number of resorption and implantation sites, and live and dead fetuses, and live fetal body weight were recorded. Fetuses were examined for skeletal and soft tissue abnormalities by gross examination and alizarin red S dye staining.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: The author concluded “The administration of up to 175 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.”

Reliability: (1) valid without restriction
Although description of protocol was limited and the results were tabulated, the study was conducted for the FDA and was considered reliable.
28-MAR-2001 (20)

Species: rabbit **Sex:** male
Strain: other: Dutch-belted
Route of administration: other: Oral intubation
Exposure period: Gestation days 6-18
Frequency of treatment: Daily
Duration of test: 29 days
Doses: 0, 2.5, 12, 54, or 250 mg sodium benzoate/kg bw/d
Control Group: other: Sham-treated or 2.5 mg 6-aminonicotinamide/kg bw on gestation day 9 (positive control)
Result: NOAEL Developmental Toxicity: >250 mg/kg bw/d

Year: 1972
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: Fetal data with dose level: No soft tissue abnormalities were reported in any of the test groups except in the positive control group (i.e., anopia, short tail, cleft palate, and club foot). Various skeletal abnormalities (i.e., incomplete ossification of the sternebrae, bipartite, fused, and extra sternebrae, and craniostosis) were reported in all test groups including sham-treated controls, but there were no differences between sodium benzoate-treated rabbits and sham-treated controls.
Maternal data with dose level: No significant differences between control and sodium benzoate-treated rabbits were reported; however, resorptions (i.e., total number, percent partial resorptions, and percent complete resorptions) appeared to be higher only in the 54 mg/kg bw/d dose group.
NOAEL Maternal Toxicity: >250 mg/kg bw/d
Test condition: Groups of 10-12 pregnant Dutch-belted rabbits were administered 0, 2.5, 12, 54, or 250 mg sodium benzoate/kg bw/d by oral intubation as a water solution during gestation days 6 to 18 inclusive. Ten pregnant rabbits were administered 2.5 mg 6-aminonicotinamide/kg bw on gestation day 9 and served as the positive control group. Appearance, behavior, and food consumption were monitored daily. Body weights were recorded on gestation days 0, 6, 12, 18, and 29. On gestation day 29, dams underwent Caesarean section. The number of resorption and implantation sites, and live and dead fetuses, and live fetal body weight were recorded. Fetuses were examined for skeletal and soft tissue abnormalities by gross examination and alizarin red S dye staining.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)
Conclusion: The author concluded “The administration of up to 250 mg/kg

(body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Reliability: (1) valid without restriction
Although description of protocol was limited and the results were tabulated, the study was conducted for the FDA and was considered reliable.

28-MAR-2001 (20)

Species: other: Hamster/outbred golden **Sex:** male
Strain: other: outbred golden
Route of administration: other: Oral intubation
Exposure period: Gestation days 6-10
Frequency of treatment: Daily
Duration of test: 14 days
Doses: 0, 3, 14, 65, or 300 mg sodium benzoate/kg bw/d
Control Group: other: Sham-treated or 250 mg aspirin/kg bw/d (positive control)
Result: NOAEL Developmental Toxicity: >300 mg/kg bw/d

Year: 1972
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not reported
Result: Fetal data with dose level: No soft tissue abnormalities were reported in any of the test groups. Various skeletal abnormalities (i.e., incomplete ossification of the sternebrae, bipartite and missing sternebrae, wavy ribs, incomplete ossification of the vertebrae and extremities, and missing or reduced hyoid) were reported in all test groups including controls, but there were no differences between sodium benzoate-treated hamsters and controls.
Maternal data with dose level: No significant differences between control and sodium benzoate-treated hamsters were reported.
NOAEL Maternal Toxicity: >300 mg/kg bw/d
Test condition: Groups of 21-22 pregnant outbred golden hamsters were administered 0, 3, 14, 65, or 300 mg sodium benzoate/kg bw/d or 250 mg aspirin/kg bw/d (positive control) by oral intubation as a water solution during gestation days 6 to 10 inclusive. Appearance, behavior, and food consumption were monitored daily. Body weights were recorded on gestation days 0, 8, 10, and 14. On gestation day 14, dams underwent Caesarean section. The number of resorption and implantation sites, and live and dead fetuses, and live fetal body weight were recorded. Fetuses were examined for skeletal and soft tissue abnormalities by gross examination and alizarin red S dye staining.
Test substance: Methyl benzoate (data for structurally related substance sodium benzoate)

Conclusion: The author concluded "The administration of up to 300 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Reliability: (1) valid without restriction
Although description of protocol was limited and the results were tabulated, the study was conducted for the FDA and was considered reliable.

28-MAR-2001 (20)

5.8.3 Toxicity to Reproduction, Other Studies

Type: other: 5-generation
In Vitro/in vivo: In vivo
Species: mouse
Strain: other: cross-bred white **Sex:** male
Route of administration: other: Oral
Exposure period: Premating exposure, Females: 8 months Premating exposure, Males: 8 months
Frequency of treatment: Daily
Duration of test: 5 generations
Doses: 40 mg benzoic acid/kg bw/d or 40 mg benzoic acid/kg bw/d in conjunction with 80 mg sodium bisulphite/kg bw/d
Control Group: other: Basal diet only

Method: other: Reproductive toxicity
Year: 1970
GLP: no
Test substance: other TS

Remark: Statistical evaluations: No
Result: Offspring toxicity F1 and F2: In the reproduction phase of the study, body weight gain at 3.5 months in the benzoic acid/sodium bisulphite combination-fed mice was less in the F1 and F2 generation, but was similar or exceeded controls in the F3 and F4 generations.
Parental data and F1: Results were not clearly reported for all parameters examined; however, it appears that survival was reduced in mice fed the benzoic acid/sodium bisulphite combination. Benzoic acid-fed mice and benzoic acid/sodium bisulphite combination-fed mice were more sensitive than controls to mortality (50 and 51.5% for treated mice versus 12.5% for controls) and weight loss (26 and 22.4% for treated mice versus 17.8% for controls) after 100% food restriction. Results for the swimming test of treated versus control were not reported. There did not appear to be an effect on organ weights relative to body weight.
Test condition: For a period of 17 months, groups of 25 male and 25 female cross-bred white mice were fed 40 mg benzoic acid/kg bw/d in

a paste prior to normal feeding. Similarly, groups of 25 male and 25 female cross-bred white mice were fed 40 mg benzoic acid/kg bw/d in conjunction with 80 mg sodium bisulphite/kg bw/d. A control group received basal diet only. Animals were observed for general condition, behaviour and survival. Food consumption and body weight gain were recorded and the animals were tested to determine the possible effects of hunger and physical stress (swimming test). At study termination, organ weights were recorded. In addition, after 8 months of treatment, some benzoic acid/sodium bisulphite combination-fed mice were mated to study effects on reproduction over 5 generations. Body weight gain of weaned pups was recorded for 3-5 months.

Test substance: Methyl benzoate (data for structurally related benzoic acid)

Conclusion: Benzoic acid administration appeared to increased sensitivity of mice to mortality, but no effect on reproduction was apparent. There were no statistical analyses conducted and the results were not clearly reported.

Reliability: (3) invalid
Methodology was unconventional and there was only use of 1 dose. No statistical analyses were conducted and results were not clearly described.

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IUCLID Data Set

Existing Chemical	ID: 99-75-2
CAS No.	99-75-2
EINECS Name	methyl p-toluate
EC No.	202-784-1
Molecular Formula	C9H10O2

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo:	Benzyl Derivatives
Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 33.2 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

Value: = 34 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (2)

2.2 Boiling Point

Value: = 220 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

2.4 Vapour Pressure

Value: = .14 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a measured boiling point of 220 C.
Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (12)

2.5 Partition Coefficient

log Pow: = 2.38 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (11)

log Pow: = 2.7 at 25 degree C

Method: other (measured)
Year: 1993
GLP: no data

Method: Measured
Test substance: Methyl p-methylbenzoate
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (5)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 374.5 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a log Kow = 2.70
Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (13)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 64.5 hour(s)

GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (7)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: other TS

Result: 1.1 years at pH 8 and 11years at pH 7
Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (10)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 363000
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44% Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (6)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 19.7
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44% Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable

The data are obtained by a recognized fugacity calculation method. Data are considered

16-NOV-2001 (6)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 61.6
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44%
Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (6)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.0015
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44%
Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, MP & calculated water solubility, VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (6)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 25.1
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44%
Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (6)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 9.86
Aerosol =0.00025% Air =34.6% Fish =0.0011% Sediment =0.44%
Soil =20.0% Suspended Sediment =0.014% Water =45.0%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation

method. Data are considered

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 0.90; nonlinear - 0.996. Expert survey results: ultimate - 2.9 weeks; primary - 3.8 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Conclusion: Methyl p-methyl benzoate is predicted to be readily degradable.
Reliability: (2) valid with restrictions
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (8)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Result: 96 hour LC50 = 9.6 mg/L
Test substance: Methyl p-methylbenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (9)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Conclusion: 48 hour LC50 = 28.0 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (9)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: mg/l **Analytical monitoring:** no data
EC10: - calculated
EC50: = .78 -

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Methyl p-methylbenzoate
Conclusion: 96 hour EC50 = 0.78 mg/L
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (9)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data

Method: other: LD50 calculated, dose range is 95 confidence interval
Year: 1977
GLP: no
Test substance: other TS

Remark: No effects at lowest dose with slight lethargy at 1.73 g/kg bw, ataxia, piloerection, ptosis, and lethargy at 2.47 g/kg bw, and flaccid, lethargy and coma at the highest dose.
Result: LD50 = 3300 (2200-5000) mg/kg bw (95% Confidence Limit)
Number of deaths at each dose level: 0/10, 1/10, 2/10, and 10/10 deaths at 1220, 1730, 2470, and 5000 mg/kg bw, respectively
Test condition: Rats were orally administered (method not stated) 1.22, 1.73, 2.47 or 5.0 g/kg bw of test substance
Test substance: Methyl p-methylbenzoate
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results.Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (4)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data

Method: other: LD50 calculated, dose range is 95 confidence interval
Year: 1977
GLP: no
Test substance: other TS

Remark: No toxic effects were reported. Mild to severe redness and mild to moderate edema were observed.
Result: LD50 = >5000 g/kg bw (95% confidence limit)
Number of deaths at each dose level: 0/10 deaths at 5000 mg/kg bw
Test condition: Ten rabbits were topically administered 5 g/kg bw of test substance.
Test substance: Methyl p-methylbenzoate
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results.Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (3)

5.1.4 Acute Toxicity, other Routes

-

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA97, TA98, TA1535, and TA1537
Concentration: 0, 3, 10, 33, 100, 333, 666, 1000, 1666, or 3333 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1992
GLP: yes
Test substance: other TS

Result: Methyl p-methylbenzoate showed no mutagenic activity in any of the strains tested with or without S9.
Test condition: Metabolic activation: S9 fractions of Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamster livers Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98 and 1538), mitomycin C (TA102), methyl methanesulfonate (TA104) and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains without S9. 2-Aminoanthracene was used with all strains incubated with S9 and either sterigmatocystin or 2-aminoanthracene was used for TA102. DMSO was used as the solvent control. Nine concentrations of methyl p-toluate (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 C for 48 hours. The number of revertants was machine counted. If a chemical was not active (with or without metabolic activation) in all Salmonella strains tested, it was considered non mutagenic.
Test substance: Methyl p-methylbenzoate (99+% purity)
Conclusion: Methyl p-methylbenzoate was non-mutagenic in this assay.
Reliability: (1) valid without restriction
Assay was conducted by standard methodology and published in a peer reviewed journal. Tabulated results.
07-AUG-2001 (14)

5.6 Genetic Toxicity 'in Vivo'

-

5.8.2 Developmental Toxicity/Teratogenicity

-

5.8.3 Toxicity to Reproduction, Other Studies

-

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(8) US EPA Estimation Program Interface (EPI) Suite (2000) BIOWIN v4.00, EPA and Syracuse Research Corporation.

(9) US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR v0.99g classes, EPA and Syracuse Research Corporation.

(10) US EPA Estimation Program Interface (EPI) Suite (2000) HYDROWIN v1.67, EPA and Syracuse Research Corporation.

(11) US EPA Estimation Program Interface (EPI) Suite (2000) KOWWIN v1.66, EPA and Syracuse Research Corporation.

(12) US EPA Estimation Program Interface (EPI) Suite (2000) MPBPWIN v1.40, EPA and Syracuse Research Corporation.

(13) US EPA Estimation Program Interface (EPI) Suite (2000) WSKOWWIN v1.40, EPA and Syracuse Research Corporation.

(14) Zeiger, E. Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992) Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mut., 19 (Suppl 21), 2-141.

IUCLID Data Set

Existing Chemical	ID: 119-36-8
CAS No.	119-36-8
EINECS Name	methyl salicylate
EC No.	204-317-7
TSCA Name	Benzoic acid, 2-hydroxy-, methyl ester
Molecular Formula	C8H8O3

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo: Benzyl Derivatives

Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = -8.6 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (23)

Value: = -8 degree C

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (4)

2.2 Boiling Point

Value: = 220 - 224 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (23)

Value: = 222.9 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (4)

2.4 Vapour Pressure

Value: = .12 hPa at 20 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (6)

Value: = .05 hPa at 25 degree C

Method: other (measured)
Year: 1989
GLP: no data
Test substance: other TS

Method: Measured
Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (5)

Value: = .072 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a measured boiling point of 222.9 C.
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (30)

2.5 Partition Coefficient

log Pow: = 2.55 at 25 degree C

Method: other (measured)
Year: 1994
GLP: no data

Method: Measured
Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (22)

log Pow: = 2.6 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (29)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 700 mg/l at 30 degree C

Method: other
Year: 1992
GLP: no data
Test substance: other TS

Method: Measured
Test substance: Methyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
The data are obtained from a recognized database and are consistent with chemical structure.
16-NOV-2001 (33)

Solubility in: Water
Value: = 1875 mg/l at 25 degree C
Method: other
GLP: no data
Test substance: other TS
Method: Calculated
Test condition: Calculated based on a log Kow = 2.55
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (31)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 11.6 hour(s)

GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (25)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: other TS

Result: 201 days at pH 8 and 5.5 years at pH 7
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (28)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1300000
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46% Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 14.0
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46%
Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 43.6
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46%
Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.00040
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46%
Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 17.7
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46%
Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 6.98
Aerosol =0.00035% Air =13.2% Fish =0.0012% Sediment =0.46%
Soil =20.6% Suspended Sediment =0.014% Water =65.7%
Test condition: Input parameters: MW, log Kow, water solubility, MP & calculated VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (24)

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 0.97; nonlinear - 0.997. Expert survey results: ultimate - 3.1 weeks; primary - 3.9 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (26)

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Method: other: OECD Guideline 301 Sealed vessel test (CO_2 production test)
Year: 1995
GLP: no data
Test substance: other TS

Result: Degradation % after time: 98.4% at 28 days
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Innoculum: 10% by volume of secondary effluent from an unacclimatised activated sludge
The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 17-22 C. The mean percentage biodegradation was calculated from 4 vessels on day 28
Test substance: Methyl 2-hydroxybenzoate (100% purity)
Conclusion: Methyl salicylate is classified as readily and ultimately biodegradable.
Reliability: (1) valid without restriction
The study is not confirmed to be GLP, but follows OECD guidelines and is considered reliable.
16-NOV-2001 (20)

Type: anaerobic
Inoculum: activated sludge
Result: other: Total degradation
Method: other: Chemical oxygen demand
Year: 1976
GLP: no
Test substance: other TS

Result: Degradation % after time: 98.8% at <120 hours
Kinetic: 95 mg COD/gL
Time required for 10% degradation: <120 hours
Total Degradation: Yes
Test condition: 10 day window criteria: Yes
Contact time: Up to 120 hours
Innoculum: From activated sludge
The concentration of test material is increased during activation until it reaches 200 mg/L COD. Degradation is carried out on an initial concentration equivalent to 200 mg/L COD and continues until there is no measured decrease in COD.
Test substance: Methyl 2-hydroxybenzoate (data for structurally related substance 2-hydroxybenzoate, salicylic acid)
Conclusion: Methyl 2-hydroxybenzoate is predicted to be readily degradable.
Reliability: (2) valid with restrictions
The data were obtained prior to GLP and OECD guidelines but data are consistent with chemical structure. Some detail not available but published in a peer reviewed journal.
16-NOV-2001 (19)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Result: 96 hour LC50 = 11.7 (esters) and 96 hour LC50 = 10.2 mg/L (phenols)
Test substance: Methyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (27)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Conclusion: 48 hour LC50 = 38.2 (esters) and 48 hour LC50 = 4.90 mg/L (phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (27)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Methyl 2-hydroxybenzoate
Conclusion: 96 hour EC50 = 0.95 (esters) and 96 hour EC50 = 24.6 mg/L (phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (27)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: mouse
Strain: other: ddY
Sex: male
No. of Animals: 10
Vehicle: other:Olive oil

Method: other: LD50 calculated by using the Litchfield-Wilcoxon method
Year: 1984
GLP: no
Test substance: other TS

Result: LD50 = 1,390 (1,250 - 1,540) mg/kg bw
Number of deaths at each dose level: At 1,000, 1,200, 1,300, 1,500 and 1,700 mg methyl salicylate/kg bw there were 1, 2, 4, 4, and 9 deaths, respectively.
Test condition: Groups of male mice fasted for 20 hours were administered 1,000, 1,200, 1,300, 1,500 or 1,700 mg methyl salicylate/kg bw in olive oil and observed for 7 days. The LD50 with 95% confidence intervals was calculated.
Test substance: Methyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
Although the study was translated from a foreign article and there is no indication of GLP, the description of the study indicates that the methodology used was within the current standards and therefore the data are considered reliable.
30-JUN-2001 (18)

Type: LD50
Species: rat
Strain: Osborne-Mendel
Sex: male/female
No. of Animals: 5
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by using the Litchfield and Wilcoxon method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: other TS

Remark: Slope function: 1.5 (95% C.L. 1.2-1.8). Depression was reported soon after treatment. Time of deaths was between 4 and 18 hours.
Result: LD50 = 887 mg/kg bw (95% C.L. 715-1100)
Number of deaths at each dose level: Not reported
Test condition: Five male and five female young adult Osborne-Mendel rats were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period

was up to 2 weeks.
Test substance: Methyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (9)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 5
Vehicle: other:50% alcohol in polyethylene glycol 200 v/v
Route of admin.: other: Gavage

Method: LD50 calculated by using the Hazleton protocol number P7/152 and OECD Guidelines for Testing Chemicals May 12, 1981
Year: 1982
GLP: no
Test substance: other TS

Remark: At all doses, toxicity was manifested as "piloerection, shaggy coat, hunched posture, lethargy and oscillated movements". Body weight gain was not affected in surviving animals. No pathological effects were found in surviving animals killed at the end of the study. Animals dying during the study showed "severe congestion in liver, stomach overloaded, black flakes in stomach and slight reddening on mucosal surface of corpus and antrum of stomach".

Result: LD50 = 2,823 (2,479-3,214) mg/kg bw [combined male and females]; 3,049 (2,571-3,615) mg/kg bw [males]; 2,642 (2,244-3,109) mg/kg bw [females]
Number of deaths at each dose level: At 2,500, 3,150, 3,969, and 5,001 mg/kg bw, 1, 3, 4, and 5 males died and 2, 4, 5, and 5 females died, respectively.
Test condition: Based on the results of a preliminary dose range-finding study, groups of 5 male and 5 female rats were gavaged with 2,500, 3,150, 3,969, or 5,001 mg methyl salicylate/kg bw following an overnight fast and then observed for 14 days. Body weights were measured on day 0, 7 and 14. At the end of 14 days all surviving animals were killed by CO2 inhalation and the animals were examined macroscopically.
Test substance: Methyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
This study was conducted at a reputable laboratory following OECD guidelines and therefore the data are considered reliable.
30-JUN-2001 (34)

Type: LD50
Species: other: Guinea pig
Strain: no data
Sex: male/female
Vehicle: no data

Route of admin.: other: Gavage

Method: LD50 calculated by using the Litchfield and Wilcoxon method, dose range is 95 confidence interval
Year: 1964
GLP: no
Test substance: other TS

Remark: Slope function: 1.6 (95% C.L. 1.3-1.9). Convulsions and irritated gastrointestinal tract were reported. Time of deaths was between 1 hour and 3 days.
Result: LD50 = 1060 mg/kg bw (95% C.L.873-1300)
Number of deaths at each dose level: Not reported
Test condition: Groups of guinea pigs consisting of both males and females were fasted for 18 hours prior to treatment. Animals were observed for toxic signs and death. The observation period was up to 2 weeks.
Not reported
Test substance: Methyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (10)

5.1.2 Acute Inhalation Toxicity

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5.1.3 Acute Dermal Toxicity

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 17 weeks
Frequency of treatment: daily
Post exposure period: No
Doses: 0, 0.1, 1.0% in diet Actual dose: Approximately 0, 50, or 500 mg/kg bw/d
Control Group: other: diet only

Method: other: 17-week feeding study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 1%
NOAEL: 0.1%
Toxic response/effects by dose level: Decreased body weight gain in rats of both sexes administered highest dose. No gross or microscopic effects reported at any dose level.
Test condition: Groups of 10 male and 10 female weanling rats were fed 0, 0.1 or 1% methyl salicylate in the diet for 17 weeks. Rats were killed by a blow to the head and weights of liver, kidneys, spleen and testes were recorded. The liver, kidney, spleen and testes of 4 male rats in control and 1% diet groups were examined microscopically. The liver kidney , spleen, adrenal and thyroid of 4 female rats in control and 1% diet groups were also examined microscopically.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: 1% methyl salicylate in the diet produced reduced body weight gain when administered to rats over 17 weeks.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (32)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: 2 years
Frequency of treatment: daily
Post exposure period: No
Doses: 0, 0.1, 0.5, 1, 2% methyl salicylate in the diet Actual dose: Approximately 0, 50, 250, 500, or 1,000 mg/kg bw/d
Control Group: other: diet only

Method: other: 2-yr feeding study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 0.5%
NOAEL: 0.1%
Toxic response/effects by dose level: Rats in the highest dose group exhibited increased amounts of cancellous bone tissue and fewer osteoclasts compared to controls and did not survive past day 49 of the study. At 0.5% and 1%, a slight excess of cancellous bone was reported in 1/11 and 2/11 bones examined, respectively. Growth inhibition reached statistical significance at the 2 highest dose levels. No other significant effects were reported at any dose.
Test condition: Groups of 25 male and 25 female rats were fed methyl salicylate in the diet for a period of 2 years. Hematology was conducted at 3, 11, 17 and 22 months on 10 rats at each concentration. At termination, organ weights were recorded and gross and microscopic examinations were made. Tissues examined in 108 rats included thyroid, parathyroid, lung, heart, stomach, pancreas, spleen, liver, kidney, adrenal, lymph note, small intestine, bone marrow smear, leg bone and muscle, urinary bladder, and testis and prostate or ovary and uterus.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate produced an increase in cancellous bone in all rats fed 2% which was less pronounced in rats fed 1%.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (32)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Osborne-Mendel
Route of administration: oral feed
Exposure period: up to 71 days
Frequency of treatment: daily
Post exposure period: No
Doses: 0 and 2% in the diet Actual dose: Approximately 1,000 mg/kg bw/d
Control Group: other: basal diet

Method: other: 71-day feeding study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 2%
Toxic response/effects by dose level: Of the treated animals, one male died at 11 days, 2 males died at 19 days, and the females died at days 31, 40, and 71. Controls were healthy throughout the study. Grossly, 4 out of 6 treated rats had lung damage compared to none in the controls. The x-rays showed an increased area of dense bone in growth areas of all bones particularly the long bones of the limbs. 3 out of 6 treated rats showed focal gastric hemorrhages in the glandular stomach.
Test condition: Supplemental study to examine reported changes in bone of rats fed methyl salicylate in the diet at 2%. 3 male and 3 female rats were fed 2% methyl salicylate in the diet for a period of 71 days. Full carcass x-rays were taken to examine changes in bone. A hind leg, front leg and sternum of each rat was decalcified for microscopic examination. Sections of brain, thyroid, heart, lung, liver, kidney, stomach, pancreas, duodenum and adrenal of each rat were prepared for microscopic examination.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate at 2% in the diet of rats was reported to produce increased mortality, changes in bone, and effects on lung and stomach tissue. However, the individual data for these results were not available and could not be fully interpreted.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 30 weeks
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0, 0.2, 0.36, 0.63, 1.13, or 2.0% methyl salicylate (approximately 0, 100, 180, 315, 565, or 1,000 mg/kg bw/d, respectively) Actual dose: approximately 0, 100, 180, 315, 565, or 1,000 mg/kg bw/d
Control Group: other: basal diet

Method: other: 30-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 565 mg/kg bw/d
NOAEL: 315 mg/kg bw/d
Toxic response/effects by dose level: Decreased body weight gain as a result of decreased food consumption was reported in all rats fed the 2 highest concentrations and in male rats fed 0.63% methyl salicylate. At the 2 highest dose levels, X-rays taken at week 10 showed increased bone density at the metaphyses of the femur, humerus, tibia, and radius.
Test condition: In the first of a series of 6 experiments, groups of Sprague Dawley rats (5/sex) were fed diets containing 0, 0.2, 0.36, 0.63, 1.13, or 2.0% methyl salicylate (approximately 0, 100, 180, 315, 565, or 1,000 mg/kg bw/d, respectively) for a period of 30 weeks to assess the possible osseous effects of methyl salicylate. The diets were introduced to the rats in a step-wise fashion and were fed at 100% of the concentrations by week 5.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: A second experiment was conducted to assess whetherbone lesions observed at dietary concentrations of 1.13% or higher were specific to methyl salicylate.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 11 weeks
Frequency of treatment: ad libitum
Post exposure period: vot reported
Doses: 2% in the diet Actual dose: approximately 1,000 mg/kg bw/d
Control Group: other: basal diet

Method: other: 11-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
The authors concluded that the effect on bone is related to the ortho-hydroxybenzoic acid structure.

Result: LOAEL: 2%
Toxic response/effects by dose level: All animals died in the ASA group by week 8 and by week 11 in the sodium salicylate group. Methyl salicylate-treated rats showed 80% survival and the remaining groups survived to the end of the study. Growth was depressed as compared to basal diet-only rats for groups fed methyl salicylate, ASA, and sodium salicylate. Bone lesions (i.e., increased bone density) were reported in rats fed methyl salicylate, ASA, and sodium salicylate. No effects were seen in animals fed methyl p-hydroxybenzoate or methyl m-hydroxybenzoate.

Test condition: In the second of 6 experiments, additional structurally related substances were evaluated to assess whether the bone lesions were specific to methyl salicylate. Groups of 12 Sprague Dawley rats equally divided by sex were fed 2.0% methyl p-hydroxybenzoate, 2.0% methyl m-hydroxybenzoate, 2.36% acetylsalicylic acid (ASA), or 2.1% sodium salicylate in the diet for 11 weeks. Methyl salicylate at 2% in the diet was used as a positive control (15 rats) and animals fed basal diet were used as a negative control (20 rats). Whole body X-rays were conducted periodically.

Test substance: Methyl 2-hydroxybenzoate

Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 6 weeks
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0, 0.6, or 2.0% in the diet Actual dose: Approximately 0, 300, or 1,000 mg/kg bw/d
Control Group: other: basal diet

Method: other: 6-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: None described
Result: LOAEL: 2.0%
NOAEL: 0.6%
Toxic response/effects by dose level: There were no deaths in rats fed 0 or 0.6% methyl salicylate ad libitum. Growth curves showed rats consuming 0.6% methyl salicylate ad libitum were slightly below those not receiving methyl salicylate. Mean body weight was reduced in rats fed 2.0% methyl salicylate ad libitum and mortality reached 90% by the end of the study.
Test condition: In the third of 6 experiments of the series, the nutritional implications of the reduced food intake and body weight gain reported in the first two experiments were assessed. Groups of 10 male Sprague Dawley rats were fed ad libitum 0, 0.6, or 2.0% methyl salicylate in the diet or were pair-fed (equal to the amount of food consumed by the 2.0% methyl salicylate ad libitum group) 0, 0.6% methyl salicylate or 2.0% methyl p-hydroxybenzoate for 6 weeks.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: In all cases, the pair-fed rats showed similar body weight curves and mortalities to those of rats fed 2.0% methyl salicylate ad libitum.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 12 weeks
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0.6 or 2.0% in the diet Actual dose: approximately 300 or 1,000 mg/kg bw/d
Control Group: other: no

Method: other: 12-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 2.0%
NOAEL: 0.6%
Toxic response/effects by dose level: Mortality was 100% within 8 days in both groups fed ASA, after 2 weeks in rats fed 2.1% sodium salicylate, after 7 weeks in rats fed 0.7% sodium salicylate, and within 6 weeks in rats fed 2.0% methyl salicylate. All rats fed 0.6% methyl salicylate survived to the end of the study. Whole body X-rays showed bone lesions in all high-dose treatment groups.
Test condition: The fourth of 6 experiments was conducted to assess the dietary concentration required to elicit bone lesions with ASA or sodium salicylate. Groups of 5 male Sprague Dawley rats were fed diets containing 0.6 or 2.0% methyl salicylate, 0.7 or 2.3% ASA, or 0.7 or 2.1% sodium salicylate for a period of 12 weeks.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate at 2.0% in the diet of rats produced bone lesions.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 11 weeks
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0, 0.6, 0.9, 1.2, or 2.0% in the diet Actual dose: approximately 0, 300, 450, 600, or 1,000 mg/kg bw/d
Control Group: other: basal diet

Method: other: 11-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 1.2%
NOAEL: 0.9%
Toxic response/effects by dose level: No bone lesions were observed in rats fed 0.9% methyl salicylate or less. In addition, there was a good correlation in the evaluation of bone lesions using whole body X-rays and histopathological examinations.
Test condition: In the fifth of 6 experiments, groups of Sprague Dawley rats (10/sex) were fed 0, 0.6, 0.9, 1.2, or 2.0% methyl salicylate in the diet for 11 weeks in order to determine the progression of the bone lesions and identify a more precise lowest-observed-effect level (LOEL). Each week, 2 rats from each group were X-rayed and one week following the X-rays, the same rats were killed and the femurs of some of the rats were histopathologically examined following sectioning and decalcification.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate did not produce bone lesions when fed in the diet at 0.9% or less.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 12 weeks
Frequency of treatment: ad libitum
Post exposure period: Not reported
Doses: 0 or 1.2% in the diet with and without supplemental calcium Actual dose: approximately 0 or 600 mg/kg bw/d
Control Group: other: basal diet

Method: other: 12-week feeding study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: At the end of the study, mortality was increased in treated rats not supplemented with calcium carbonate (methyl salicylate: 80-100% no calcium, 10-20% with calcium; ASA: 78% no calcium, 0% with calcium; controls: 0-20% no calcium). No bone lesions were reported in any rat supplemented with calcium carbonate. Bones lesions were reported after 3 weeks in rats fed 1.2% ASA alone. Mean body weights were similar to controls when treated rats were supplemented with calcium.
Test condition: The last of 6 experiments was conducted to determine the effect of supplemental calcium on bone lesions in rats fed methyl salicylate or ASA. Over a 12-week period, groups of 5-10 male and female Sprague Dawley rats were fed 0 or 1.2% methyl salicylate or ASA with and without supplemental calcium (0.3% for methyl salicylate and 0.36% for ASA, as calcium carbonate).
Test substance: Methyl 2-hydroxybenzoate
Conclusion: The authors concluded that supplementation with approximately 3% calcium carbonate helped prevent formation of bone lesions and the reduction of growth and survival.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: rabbit **Sex:** male
Strain: no data
Route of administration: other: dermal
Exposure period: 28 days
Frequency of treatment: daily, 5 days/week
Post exposure period: No
Doses: 0.5, 1, 2, or 4 ml/kg bw/d
Control Group: other: none

Method: other: 28-day dermal study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 2 ml/kg bw/d
NOAEL: 1 ml/kg bw/d
Toxic response/effects by dose level: Increased mortality was reported at 4 ml/kg bw/d with skin lesions at 2 ml/kg bw/d.
Test condition: Methyl salicylate was dermally applied to the clipped back of groups of 3 male and 3 female rabbits at doses of 0.5, 1, 2, or 4 ml/kg bw/d 5 days/week for 28 days. At termination, gross and microscopic examinations were conducted.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: No skin abnormalities were reported in rabbits dermally exposed to 1 ml/kg bw/d or less.
Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.
04-MAR-2001 (32)

Type: Sub-acute
Species: other: Dog **Sex:** male
Strain: no data
Route of administration: other: oral
Exposure period: 59 days
Frequency of treatment: daily, 6 days/week
Post exposure period: No
Doses: 0, 50, 100, 250, 500, 800, or 1,200 mg/kg/d
Control Group: other: 0 mg/kg/d

Method: other: 59-day oral toxicity study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 500 mg/kg bw/d
NOAEL: 250 mg/kg bw/d
Toxic response/effects by dose level: Dogs in 2 highest dose groups vomited following test substance administration.

Dogs in the 500 mg/kg/d group had diarrhea and weakness. The dogs in these 3 highest dose groups died within one month of treatment or were killed in extremis. Except for fatty liver in one dog of the 2 highest dose groups, no other microscopic effects were reported.

Test condition: Groups of 1 male and 1 female dog were orally administered a capsule containing 0, 50, 100, 250, 500, 800, or 1,200 mg methyl salicylate/kg/d 6 days/week for 59 days. At termination, liver and kidney sections and bone smears were taken from all animals. The thyroid, kidney, spleen, adrenal, bone, skeletal muscle, mesenteric lmph node, gallbladder, pancreas and testis and prostate or ovary and uterus were microscopically examined from one dog of the 800 mg/kg bw/d group and from 2 dogs of the 250 mg/kg bw/d group.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: Doses of 500 mg/kg bw/d or higher in dogs were not well tolerated causing fatty liver, diarrhea, vomiting and eventual death. No adverse effects were reported at doses up to 250 mg/kg bw/d over 59 days.

Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (32)

Type: Sub-acute
Species: other: Dog/Beagle **Sex:** male
Strain: other: Beagle
Route of administration: other: oral
Exposure period: 2 years
Frequency of treatment: daily, 6 days/week
Post exposure period: No
Doses: 0, 50, 150, or 350 mg/kg bw/d
Control Group: other: 0 mg/kg bw/d

Method: other: 2-year oral toxicity study
Year: 1963
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 150 mg/kg bw/d
NOAEL: 50 mg/kg bw/d
Toxic response/effects by dose level: Retarded growth and enlarged liver were reported at the 2 highest doses. No effects were reported on hematology.

Test condition: Groups of 2 male and 2 female beagles were orally administered 0, 50, 150, or 350 mg methyl salicylate/kg bw/d in capsules, 6 days/week for 2 years. One high-dose female was replaced for hepatitis after 33 days; the replacement was replaced for canine distemper after 19 weeks. Hematology was conducted prior to the treatment, at 2 weeks, 1 month, 3 months, 6 months, 1 year and 2 years. Necropsies

were conducted on all animals and microscopic examinations were conducted on surviving high-dose animals with limited microscopic examinations on the remaining dogs.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: Doses of 50 mg methyl salicylate /kg bw/d or less in dogs over a period of 2 years showed no adverse effects. At higher doses, up to 350 mg/kg bw/d, retarded growth and enlarged livers were reported.

Reliability: (2) valid with restrictions
This study was performed by the Food and Drug Administration prior to the establishment of GLP and OECD. Data are considered reliable.

04-MAR-2001 (32)

Type: Sub-acute
Species: other: Dog/Beagle **Sex:** male
Strain: other: Beagle
Route of administration: other: oral
Exposure period: 7.5 months
Frequency of treatment: daily
Post exposure period: Not reported
Doses: 150, 300, 500 or 800 mg/kg bw/d in 2 divided doses
Actual dose: 150, 300, 500 or 800 mg/kg bw/d
Control Group: other: yes, but not described

Method: other: 7.5-month oral toxicity study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: LOAEL: 500 mg/kg bw/d
NOAEL: 300 mg/kg bw/d
Toxic response/effects by dose level: All animals in the control and 2 lowest dose groups survived to the end of the study. At 500 mg/kg bw/d, 2 out of 6 dogs survived to the end of the study period. At the highest dose, all animals were dead by the second week. Body weight was not affected in the 2 lowest dose groups and 1 dog given 500 mg/kg bw/d showed a slight loss in body weight. Hematological analyses and urinalyses conducted on control dogs and dogs from the 2 lowest dose groups compared favorably. No such analyses were conducted on the dogs from the higher dose groups. There were no gross findings and the only organ weight findings were dose-related increases in absolute and relative liver and kidney weights of all treated dogs with the exception of dogs from the 300 mg/kg bw/d dose group enduring a reversal period of 1.5 months. The enlargement of the liver and kidneys was not accompanied by histopathological changes with the exception of extremely subtle changes in the liver indicative of increase in liver cell size and cytoplasmic granul

Test condition: Groups of 3 male and 3 female Beagle dogs were orally administered 150, 300, 500 or 800 mg methyl salicylate/kg bw/d in 2 divided doses by capsule, 6 days/week for up to

7.5 months. Control groups consisted of 2 males and 4 females. At 6.5 months some animals from the control and 2 lowest dose groups were killed and necropsied and the remaining animals in the 300 mg/kg bw/d dose group discontinued treatment with methyl salicylate. At 7.5 months, the remaining low-dose dogs and the 500 mg/kg bw/d dose group were killed and necropsied. At 8 months the dogs from the 300 mg/kg bw/d dose group that discontinued methyl salicylate treatment were killed and necropsied. All terminated dogs were subjected to organ weight evaluation and gross and histopathological examination.

Test substance: Methyl 2-hydroxybenzoate

Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

11-MAR-2001 (1)

Type: Sub-acute
Species: other: Dog/Beagle **Sex:** male
Strain: other: Beagle
Route of administration: other: oral
Exposure period: 6 months
Frequency of treatment: daily
Post exposure period: 2 months
Doses: 0, 50, 100, or 167 mg/kg bw/d
Control Group: other: yes, but not described

Method: other: 6-month oral toxicity study
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described

Result: Toxic response/effects by dose level: Survival to the end of the study was 100%. Many dogs in the test groups developed signs of seborrhea oleosum and pyoderma after 2 months, which was corrected with the addition of lard to the diet. Hematological analyses were comparable among treatment and control groups. At the 6-month necropsy, the only compound-related gross observations were changes in the gastric mucosa including gastric hemorrhage in dogs given the highest dose of ASA. Histopathological examination and relative weights of the liver and kidneys showed no differences between treated and control dogs.

Test condition: Groups of 8-12 Beagle dogs equally divided by sex were orally administered 0, 50, 100, or 167 mg/kg bw/d of methyl salicylate or ASA by capsule in 2 divided doses for a period of 6 months. All animals were killed at 6 months with the exception of 2 male and 2 female dogs from the high-dose and control groups which discontinued treatment and were maintained on basal diets for an additional 2 months. Organ weight and histopathological assessment were limited to liver, kidney and any observed lesions.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: Methyl salicylate orally administered to Beagle dogs did not

produce adverse effects at up to 167 mg/kg bw/d.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.
11-MAR-2001 (1)

Type: Sub-acute
Species: other: Human **Sex:** male
Strain: no data
Route of administration: other: oral
Exposure period: up to 14 years
Doses: 100 to 3,240 mg/person
Control Group: other:

Method: other: case studies
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: There was no indication that prolonged salicylate therapy causes bone lesions in children.
Test condition: Six clinicians conduced a retrospective evaluation of young actively growing children treated with salicylates for various forms of juvenile rheumatoid arthritis. Daily salicylate doses ranged from 100 to 3,240 mg and treatment ranged from several months to 14 years. Periodic X-rays were available for evaluating any possible bone lesions. One hundred and fifty five (155) cases were assessed.
Test substance: Methyl 2-hydroxybenzoate (salicylates medicinal)
Conclusion: Chronic salicylate therapy did not cause bone lesions in children.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.
16-NOV-2001 (1)

Type: Sub-acute
Species: other: Human **Sex:** male
Strain: no data
Route of administration: other: oral
Exposure period: up to 10 years
Doses: up to 4800 mg/kg bw/d
Control Group: other:

Method: other: case studies
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Not described
Result: Toxic response/effects by dose level: No hepatic effect or effect on growth.
Test condition: A study was conducted to evaluate a possible association between large daily doses of salicylates and hepatomegaly in children. Two hundred and eighteen (218) case studies in which up to 4,800 mg/d for periods of up to 10 years were evaluated
Test substance: Methyl 2-hydroxybenzoate (salicylates medicinal)
Conclusion: It was concluded that salicylate therapy does not cause hepatomegaly or effect growth or weight gain. Hepatomegaly was noted occasionally prior to treatment or during the course of treatment, but was considered a “concurrent happening”.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.
11-MAR-2001 (1)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 0, 1, 3.3, 10, 33.3, 100, or 333 ug/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay (Haworth et al., 1983)
Year: 1986
GLP: yes
Test substance: other TS

Result: Methyl 2-hydroxybenzoate produced no increased incidence of mutation as compared to the vehicle controls, either with or without S9 mix.
Test condition: Metabolic activation: rat liver microsome fraction S9
Sodium azide (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98), and 9-aminoacridine (TA97 and TA1537) were used as positive controls for the specific Salmonella strains

without S9. 2-Aminoanthracene was used with all strains incubated with S9. Solvent controls were also prepared concurrently. Preliminary tests were conducted to assess the cytotoxicity of the test compound and establish suitable concentrations for testing. At least 5 concentrations of the test chemicals (in triplicate) were incubated with or without S9 for 20 minutes after which plates were prepared and incubated at 37 C for 48 hours. A test chemical was considered “mutagenic” if there was a dose-related, reproducible increase in the number of revertants over background (not required to be 2-fold increase), “non mutagenic” if there was no increase, and “questionable” if there was no clear reproducible dose-related increase or “when the response was of insufficient magnitude to support a determination of mutagenicity”.

Test substance: Methyl 2-hydroxybenzoate (98% purity)
Conclusion: Methyl 2-hydroxybenzoate was non-mutagenic.
Reliability: (1) valid without restriction
NTP study

04-APR-2001 (14)

Type: other: clastogenic assay
System of testing: non bacterial Human embryo fibroblast cells
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Siser chromatid exchange
Year: 1980
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate was negative with or with out S9 activation.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.

04-APR-2001 (11)

Type: Chromosomal aberration test
System of testing: non bacterial human embryo fibroblast cells
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations
Year: 1980
GLP: no
Test substance: other TS

Result: Hydroxybenzoic acid methyl ester was negative with or with out S9 activation.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate produced positive results in this assay without metabolic activation but based on weight of evidence, the authors did not consider methyl salicylate to have mutagenic or carcinogenic potential.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
04-APR-2001 (11)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis (strain not specified)
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: with and without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1980
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate was negative with or with out S9 activation.
Test condition: Metabolic activation: with and without S9
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate was non mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
04-APR-2001 (11)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster lung fibroblasts
Concentration: not reported
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations
Year: 1980
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate was positive without S9 activation.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate produced an increase in chromosomal aberrations without S9 activation.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
04-APR-2001 (11)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98, TA100, TA1535, and TA1537
Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1975
GLP: no
Test substance: other TS

Result: 2-Hydroxybenzoic acid produced negative results.
Test condition: Metabolic activation: S9 from liver extract of Aroclor 1254-induced rats
Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)
Conclusion: 2-Hydroxybenzoic acid was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Data were collected prior to GLP and OECD guidelines but by a method considered standard and under the direction fo a recognized research institute. Therefore the data are considered reliable.
04-JUL-2001 (13)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100 and TA98
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: other TS

Result: 2-Hydroxybenzoic acid produced negative results.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)
Conclusion: 2-Hydroxybenzoic acid was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (11)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis (strain not specified)
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1980
GLP: no
Test substance: other TS

Result: 2-Hydroxybenzoic acid produced negative results.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)
Conclusion: 2-Hydroxybenzoic acid was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (11)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, and TA98
Concentration: maximum concentration = 10 mg/plate
Cytotoxic Concentration: not reported
Metabolic activation: with
Result: positive

Method: other: Ames assay
Year: 1984
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate produced negative results in all the strains tested.
Test condition: Metabolic activation: S9 fraction from liver of PCB-induced Fischer rats
Overnight cell cultures were preincubated at 37 C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control.
Test substance: Methyl 2-hydroxybenzoate (99% purity)
Conclusion: Methyl 2-hydroxybenzoate was non mutagenic.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-JUL-2001 (8)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100 and TA98
Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Ames assay
Year: 1980
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate produced negative results.
Test condition: Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (11)

Type: Chromosomal aberration test
System of testing: non bacterial Chinese hamster fibroblast cell line
Concentration: maximum concentration = 0.25 mg/ml
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: positive

Method: other: Chromosomal aberrations (Ishidate and Odashima, 1977)
Year: 1984
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate did not induce chromosomal aberrations.
Test condition: Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was >10%, equivocal if between 5.0 and 9.9%, and negative if <4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”.
Metabolic activation: none
Test substance: Methyl 2-hydroxybenzoate (99% purity)
Conclusion: Methyl 2-hydroxybenzoate was not clastogenic in this assay.
Reliability: (2) valid with restrictions
Assay was conducted by standard methodology and published in a peer reviewed journal, but the tabulated results had limited description.
04-JUL-2001 (7)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98 and TA100
Concentration: 0.1 mg/disc
Metabolic activation: with
Result: positive

Method: other: Ames assay (modified)
Year: 1992
GLP: no
Test substance: other TS

Result: The number of revertants went from 38 His+ revertants/plate in the DMSO control to 74 His+ revertants/plate in TA98

treated with methyl salicylate and S9 from hamster liver. Similarly, the number of revertants in TA100 treated with methyl salicylate and hamster S9 went from 182 to 400 His+ revertants/plate. The number of revertants were not affected in TA98 or TA100 treated with methyl salicylate without S9 or with S9 from the other animal species.

Test condition: Metabolic activation: S9 from livers of PCB-treated male SD rats, male ddY mice, male golden hamsters, or male Hartley guinea pigs.
Methyl salicylate was preincubated with the tester strain at 37 C for 30 min in a shaking water bath. After plating onto petri dishes containing Vogel-Bonner E medium, the dishes were incubated for 48 hours and revertants were counted. Induction of more than 2X the number of spontaneously occurring revertants was considered positive. Positive controls used were furyl furamide, benzopyrene, and 4-nitroquinoline-1-oxide.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: Methyl 2-hydroxybenzoate only produced positive results in TA98 and TA100 when S9 mix from hamster liver was added.

Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (12)

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA98 and TA100
Concentration: 0.1 mg/disc
Metabolic activation: with
Result: positive

Method: other: Ames assay (modified)
Year: 1992
GLP: no
Test substance: other TS

Result: 2-Hydroxybenzoic acid produced negative results in TA98, but the number of revertants went from 160 His+ revertants/plate in the DMSO control to 446 His+ revertants/plate in TA100 treated with salicylic acid and S9 from rat liver. The number of revertants were not affected in TA100 treated with salicylic acid without S9 or with S9 from the other animal species.

Test condition: Metabolic activation: S9 from livers of PCB-treated male SD rats, male ddY mice, male golden hamsters, or male Hartley guinea pigs.
Salicylic acid was preincubated with the tester strain at 37 C for 30 min in a shaking water bath. After plating onto petri dishes containing Vogel-Bonner E medium, the dishes were incubated for 48 hours and revertants were counted. Induction of more than 2X the number of spontaneously occurring revertants was considered positive. Positive

controls used were furyl furamide, benzopyrene, and 4-nitroquinoline-1-oxide.

Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)

Conclusion: 2-Hydroxybenzoic acid only produced positive results in TA100 when S9 mix from rat liver was added. Negative effects were reported with TA98.

Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (12)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 5 mg/disc
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay (Kada and Sadaie, 1972)
Year: 1992
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate produced negative results.
Test condition: After plating on petri dishes, plates were held at 4 C for 24 hours followed by a 24 hour incubation at 37 C. Afterwards, the length of the inhibition zone was measured. An inhibition zone of >2mm difference between growth inhibition zones for Rec+ and Rec- was considered to be indicative of DNA damage.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (12)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis H17(Rec+) and M45(Rec-)
Concentration: 5 mg/disc
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay (Kada and Sadaie, 1972)
Year: 1992
GLP: no
Test substance: other TS

Result: Sodium 2-hydroxybenzoate produced negative results.
Test condition: After plating on petri dishes, plates were held at 4 C for 24 hours followed by a 24-hour incubation at 37 C. Afterwards, the length of the inhibition zone was measured. An inhibition zone of >2mm difference between growth inhibition zones for Rec+ and Rec- was considered to be indicative of DNA damage.
Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)
Conclusion: Sodium 2-hydroxybenzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.
03-JUL-2001 (12)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis H17(Rec+) and M45(Rec-)
Concentration: 5 mg/disc
Metabolic activation: no data
Result: positive

Method: other: Bacillus subtilis recessive assay (Kada and Sadaie, 1972)
Year: 1992
GLP: no
Test substance: other TS

Result: 2-Hydroxybenzoic acid produced positive results with a zone difference of 4.7 mm.
Test condition: After plating on petri dishes, plates were held at 4 C for 24 hours followed by a 24-hour incubation at 37 C. Afterwards, the length of the inhibition zone was measured. An inhibition zone of >2mm difference between growth inhibition zones for Rec+ and Rec- was considered to be indicative of DNA damage.
Test substance: Methyl 2-hydroxybenzoate (data on hydrolysis product, 2-hydroxybenzoic acid)
Conclusion: The authors concluded that 2-hydroxybenzoic acid has weak DNA damaging potential.
Reliability: (2) valid with restrictions

Although this study was not conducted under GLP, the methodology used is standard and the study is published with reasonable detail. Therefore the data are considered reliable.

03-JUL-2001 (12)

Type: other: reverse mutation test
System of testing: bacterial Bacillus subtilis M45 (Rec-) and H17 (Rec+)
Concentration: 23 ug/disk
Metabolic activation: without
Result: positive

Method: other: Bacillus subtilis recessive assay
Year: 1978
GLP: no
Test substance: other TS

Result: Methyl 2-hydroxybenzoate produced negative results.
Test condition: Metabolic activation: none
The results were considered negative if the zone of inhibition was <2 mm, weakly positive if greater than or equal to 2 mm, and positive if greater than or equal to 5mm.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl 2-hydroxybenzoate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to GLP or OECD guidelines and the majority of the article was in Japanese (English summary tables), the study appeared to follow standard methodology. Therefore the data were considered reliable.

04-JUL-2001 (17)

5.6 Genetic Toxicity 'in Vivo'

Type: other: DNA damage
Species: other: Rat/Wistar **Sex:** male/female
Route of admin.: unspecified
Exposure period: Single dose
Doses: 700 mg/kg bw
Result: ambiguous

Method: other: Chromosomal aberrations
Year: 1982
GLP: no
Test substance: other TS

Test condition: Following exposure, rats were examined for "changes in the chromosome apparatus of somatic and embryonal cells".
Test substance: Methyl 2-hydroxybenzoate (data for structurally related substance sodium salicylate)
Conclusion: At a dose of 700 mg/kg bw, sodium salicylate was reported to induce both numerical and structural chromosomal aberrations in somatic and embryonic cells.

Reliability: (4) not assignable
The data were presented as a brief English summary of a Russian article and due to the limited description, it was not possible to ascertain the validity of the test methodology.
09-JUL-2001 (21)

Type: other: Mutation
Species: other: Silk worm **Sex:** no data
Route of admin.: unspecified
Exposure period: Not reported
Doses: Not reported
Result: negative

Year: 1980
GLP: no
Test substance: other TS

Remark: Methyl salicylate produced negative results.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate was non-mutagenic in this assay.
Reliability: (2) valid with restrictions
Although the data were collected prior to the establishment of GLP or OECD guidelines, the methodology used was standard and the project was overseen by the Japanese Ministry of Health and Welfare. Therefore the data were considered reliable.
03-JUL-2001 (11)

5.8.2 Developmental Toxicity/Teratogenicity

-

5.8.3 Toxicity to Reproduction, Other Studies

Type: other: 3-generation study described by Fitzhugh (1968)
In Vitro/in vivo: In vivo
Species: rat
Strain: Osborne-Mendel **Sex:** male
Route of administration: other: Diet
Exposure period: Premating exposure, Females: 100 days Premating exposure, Males: 100 days
Frequency of treatment: Daily
Duration of test: 100 days pre-mating through gestation. F1 offspring continued treatment and were mated to produce the F2 generation.
Doses: 0, 500, 1,500, 3,000 and 5,000 ppm fed ad libitum in the diet Actual dose: Approximately 0, 25, 75, 150, or 250 mg/kg bw/d, respectively
Control Group: other: Control animals received diet alone.
Result: NOEL: 1,500 ppm LOEL: 3,000 ppm

Method: other: Reproductive toxicity
Year: 1971
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. A 2-sided t-test was used to determine differences between controls and treated rats for average litter size, viability, survival, and weanling data.
Result: Offspring toxicity F1 and F2: The statistically significant changes reported in the F1 generation were not reported in the F2 generation although there was a decreasing trend observed. There were no visible abnormalities and necropsies of the F2 generation showed no effects. In the supplementary study, rats supplemented with 1500 ppm calcium carbonate showed statistically significant decreases in the number born and liveborn, survivors to day 4 and number weaned at 3000 ppm methyl salicylate. At the highest dose of 5000 ppm methyl salicylate, calcium supplemented rats \ber born and liveborn.
Parental data and F1: There was no effect on fertility index at any dose. Statistically significant effects were reported as follows: decreases in average litter size in the F1 generation at 3000 ppm (F1b) and 5000 ppm (F1a and F1b); decreases in average number of liveborn per female at the two highest doses (F1a and F1b); decreased survival at the two highest doses (F1a and F1b). External examination showed no gross abnormalities.
Test condition: Groups of 20 male and 20 female Osborne-Mendel rats were fed methyl salicylate 100 days prior to mating. The fertility of the mating pairs was recorded. In some cases due to non-treatment-related causes the groups of 20 were reduced. Litters exceeding 10 pups by day 4 were reduced to 10. The parental rats were mated 2x to produce 2 litters-F1a and F1b generations. Pups of were killed at weaning except for

selected groups of 20 pairs from the F1b generation which were used to produce a F2 generation. The same procedure was used to produce a 3rd generation with the exception that all pups, even from the F3b generation, were killed at weaning.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: At dietary concentrations up to 1500 ppm, rats showed no statistically significant effects. Fertility and mating were not affected by methyl salicylate administration. At the higher doses of 3000 and 5000 ppm, some reproductive parameters were significantly decreased. These findings were significant only in the F1 generation. Supplementation with calcium did not appear to alleviate or enhance the reported effects.

Reliability: (1) valid without restriction
Although the studywas conducted prior to GLP and OECD guidelines, it was conducted under conditions considered comparable to present standards and was published in a peer-reviewed journal.

16-MAY-2001 (3)

Type: other: 3-generation
In Vitro/in vivo: In vivo
Species: rat
Strain: Wistar **Sex:** male
Route of administration: other: Diet
Exposure period: Premating exposure, Females: 60 days Premating exposure, Males: 60 days
Frequency of treatment: Ad libitum
Duration of test: 3 generations
Doses: 0, 0.25, or 0.5% in the diet
Control Group: other: Basal diet

Method: other: Reproductive toxicity
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Results evaluated with analysis of variance and t-tests

Result: Offspring toxicity F1 and F2: No gross abnormalities were reported in any of the litters.
Parental data and F1: No statistically significant differences among treated and control animals were reported for any reproductive parameters examined including mating performance, reproductive performance, number of stillborn, viability, mean litter size, number born, number liveborn, and number live at 5 days.

Test condition: Groups of 25 male and 25 female Wistar rats (F0 generation) were fed diets containing 0, 0.25, or 0.5% methyl salicylate for 60 days after which rats were mated to produce F1a litters. Thirty days following weaning of the F1a pups, F0 females were mated to produce F1b litters. At about 4 months of age, 30 male and 30 female rats were randomly selected to produce F2a and F2b litters. All rats were

maintained on their respective test diets throughout the study.
Test substance: Methyl 2-hydroxybenzoate
Conclusion: Methyl salicylate produced no effects on reproduction at dietary concentrations of up to 0.5%.
Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.
11-MAR-2001 (2)

Type: other: Reproduction
In Vitro/in vivo: In vivo
Species: mouse
Strain: other: CD-1 **Sex:** male
Route of administration: gavage
Exposure period: Premating exposure, Females: 7 days Premating exposure, Males: 7 days
Frequency of treatment: Daily
Duration of test: over 100 days
Doses: 0, 50, 100, 250, 500, or 1000 mg/kg bw/d
Control Group: other: Control groups (40 mice/sex) were administered vehicle only.

Method: other: NTP's Fertility Assessment by Continuous Breeding (FACB)
Year: 1984
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Cochran-Armitage test, Chi-square test, Fisher's exact test, Kruskal-Wallis test, Jonckheere's test, Mann-Whitney U test, t test, and F test.
Result: Offspring toxicity F1 and F2: At the highest dose in task (2), there was a significant decrease ($p<0.05$) in the mean number of litters, the average number of pups/litter, the proportion of pups born alive, and mean live pup weights. In task (3), parameters measured (i.e., number of live pups/litter, proportion of pups born alive, sex of pups born alive, and live pup weights) were assessed yet the authors could not determine which sex was affected by methyl salicylate treatment.
Parental data and F1: In task (1), body weight was not affected; however, some animals died during treatment (2 controls, 2 at 0.05 g/kg bw/d, and 3 at 1.0 g/kg bw/d). Gavage error was attributed to 1 out of 2 deaths in controls, 2 out of 2 deaths at 0.05 g/kg bw/d and 1 out of 3 deaths at 1.0 g/kg bw/d. The other deaths were diagnosed as pulmonary congestion or cardiac myodegeneration and tubular nephrosis. In task (2), there were no compound-related deaths nor were there any clinical signs of toxicity. Body weights were not affected by treatment. The fertility index was similar in all groups and ranged from 95 to 100%. All groups in task (3), including controls, had similar, yet poor, fertility.

Test condition: The assessment consists of 4 tasks: (1) dose range-finding phase; (2) continuous breeding phase; (3) identification of the sex(s) affected by treatment; and (4) assessment of offspring. In task (1), groups of 8 male and 8 female CD-1 mice were administered 0, 0.05, 0.1, 0.25, 0.5, or 1.0 g methyl salicylate/kg bw/d in corn oil by gavage for 14 days. Based on the results of task (1), groups of 20 male and 20 female CD-1 mice were administered 0.1, 0.25, or 0.5 g methyl salicylate/kg bw/d during a 7-day premating period and throughout a 100-day cohabitation period. Controls (40 mice/sex) were administered vehicle only. In the third task, mice from the high-dose group were tested in a crossover mating trial. Treated males were mated with control females, treated females were mated with control males and controls were mated with controls (groups of 20 animals). Since there was an unusually low fertility index for controls in this task, task (3) was repeated. The study was terminated after task (3).
Test substance: Methyl 2-hydroxybenzoate (99% purity)
Conclusion: The authors concluded that methyl salicylate “does interfere with reproduction in CD-1 mice”.
Reliability: (1) valid without restriction
NTP study
11-MAR-2001 (15)

Type: other: 3-generation
In Vitro/in vivo: In vivo
Species: mouse
Strain: no data **Sex:** male
Route of administration: other: Diet
Exposure period: Premating exposure, Females: 30 days Premating exposure, Males: 30 days
Frequency of treatment: Ad libitum
Duration of test: 3 generations
Doses: 0, 0.25, or 0.5% in the diet
Control Group: other: Basal diet

Method: other: Reproductive toxicity
GLP: no
Test substance: other TS

Remark: No statistically significant differences among treated and control animals were reported for any reproductive parameters examined including mating performance, reproductive performance, number of stillborn, viability, mean litter size, number born, number liveborn, and number live at 5 days.
Statistical evaluations: Yes. Results evaluated with analysis of variance and t-tests
Result: Offspring toxicity F1 and F2: No gross abnormalities were reported in any of the litters.
Parental data and F1: No statistically significant differences among treated and control animals were reported for any reproductive parameters examined including mating

performance, reproductive performance, number of stillborn, viability, mean litter size, number born, number liveborn, and number live at 5 days.

Test condition: Groups of 25 male and 25 female mice (F0 generation) were fed diets containing 0, 0.25, or 0.5% methyl salicylate for 30 days after which mice were mated to produce F1a litters. Thirty days following weaning of the F1a pups, F0 females were mated to produce F1b litters. At about 4 months of age, 30 male and 30 female mice were randomly selected to produce F2a and F2b litters. All mice were maintained on their respective test diets throughout the study. No gross abnormalities were reported in any of the litters.

Test substance: Methyl 2-hydroxybenzoate

Conclusion: Methyl salicylate produced no effects on reproduction at dietary concentrations of up to 0.5%.

Reliability: (2) valid with restrictions
Unpublished report without date of study and limited description of protocol and results.

10-JUL-2001 (2)

Type: other: Reproduction
In Vitro/in vivo: In vivo
Species: mouse
Strain: other: CD-1 **Sex:** male
Route of administration: gavage
Exposure period: Premating exposure, Females: 7 days Premating exposure, Males: 7 days
Frequency of treatment: Daily
Duration of test: over 100 days
Doses: 0, 25, 50 or 100 mg methyl salicylate/kg bw/d in corn oil
Control Group: other: Control groups (40 mice/sex) were administered vehicle only.
Result: NOEL: 100 mg/kg bw/d

Method: other: NTP's Fertility Assessment by Continuous Breeding (FACB)
Year: 1984
GLP: yes
Test substance: other TS

Remark: Statistical evaluations: Yes. Cochran-Armitage test, Fisher's Exact test, Kruskal-Wallis test, Jonckheere's test, Mann-Whitney U test, F test, and t test.
With respect to the observation of decreased absolute and relative reproductive tract weight, the authors noted that 2 females from the control group had reproductive tract weights that were heavier than those of the other controls that delivered pups. In the treated group, 6 females did not deliver pups and had lighter reproductive tracts on average than those treated mice giving birth. The authors suggested that this, along with the fact that there were more females with postgravid reproductive tracts in the control group than in the treated group (11/19 versus

11/17), might account for the statistical difference in reproductive tract weight between controls and treated mice and concluded that the difference was likely due to biological variation and therefore not considered biologically significant.

Result: Offspring toxicity F1 and F2: In task (4), there were no statistically significant effects on mating behavior, fertility rate, or reproductive performance. In addition, at necropsy F1 parental mice, there was no significant differences in the percent motile sperm, sperm concentration or percent abnormal sperm in the cauda epididymis in treated versus control males, there was no effect on body weight in either sex, or on absolute and relative organ weights in males and on absolute and relative pituitary and liver weights in females. However, absolute and relative brain weight was significantly increased ($p<0.05$) and absolute and relative reproductive tract weights were significantly decreased ($p<0.05$) in treated females compared to controls. There were no gross or histological effects attributable to treatment in the pituitary, testis, epididymis, prostate, seminal vesicles, ovary, oviduct, uterus, or vagina of treated mice.

Parental data and F1: In task (1), 2 females, 1 female and 1 male, and 2 females and 3 males died in the 50, 100 and 1,000 mg/kg bw/d groups, respectively. Percent lethality reached statistical significance only in the high-dose group. Percent weight gain was similar among all groups. In task (2), methyl salicylate administration had no effect on the number of pairs able to produce at least one litter, the number of litters produced per pair, the number of live pups per litter, or the proportion or sex of pups born alive. At the high dose, female live pup weight (adjusted for the total number of pups per litter) was significantly greater ($p<0.05$) than those in the 50 mg/kg bw/d group and combined live pup weight (adjusted for the total number of pups per litter) was significantly ($p<0.05$) greater than those in the 25 and 50 mg/kg bw/d groups. These differences, however, were considered by the authors to be "due to chance alone". One parental male and female, 1 parental female, 2 parental females and 3 parental males, and 3 parental males from the control,

Test condition: The assessment consists of 4 tasks: (1) dose range-finding phase; (2) continuous breeding phase; (3) identification of the sex(s) affected by treatment; and (4) assessment of offspring. In task (1), groups of 8 male and 8 female CD-1 mice were administered 0, 50, 100, 250, 500, or 1000 mg methyl salicylate/kg bw/d in corn oil by gavage for 14 days. Based on the results of task (1), groups of 20 male and 20 female CD-1 mice were administered 0, 25, 50 or 100 mg methyl salicylate/kg bw/d in corn oil by gavage during a 7-day premating period, throughout a 98-day cohabitation period and 21-day segregation periods [task (2)]. Controls (40 mice/sex) were administered vehicle only. Since there was no effect in task (2), task (3) was not conducted, but

task (4) was conducted to assess the F1 offspring. One or 2 female and male pups weaned from control and high-dose mice in task (2) were randomly selected for study in task (4). The weanlings were individually housed until 90 +/- 10 days of age when they were cohabited (1 male + 1 female from different litters) fo

Test substance: Methyl 2-hydroxybenzoate (purity >99%)

Conclusion: The authors concluded that under these study conditions, "methyl salicylate (at daily oral doses as high as 100.0 mg/kg) was not a reproductive toxicant in either F0 or F1 breeding pairs of CD-1 mice."

Reliability: (1) valid without restriction
NTP study

10-AUG-2001 (16)

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IUCLID Data Set

Existing Chemical	ID: 2050-08-0
CAS No.	2050-08-0
EINECS Name	pentyl salicylate
EC No.	218-080-2
Molecular Formula	C12H16O3

Producer Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Substance Related Part

Company:	Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date:	28-JUN-2001

Memo: Benzyl Derivatives

Printing date:	16-NOV-2001
Revision date:	
Date of last Update:	**16-NOV-2001**

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 82.5 degree C

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (19)

2.2 Boiling Point

Value: = 277 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

Value: = 278 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (3)

2.4 Vapour Pressure

Value: = .004 hPa at 20 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (5)

Value: = .009 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a measured boiling point of 278 C.
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (18)

2.5 Partition Coefficient

log Pow: = 4.57 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Test substance: Pentyl 2-hydroxybenzoate (data for structurally related isomer isoamyl salicylate)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (17)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 18.94 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a log Kow = 4.57
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (20)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 7.6 hour(s)

GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (13)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: other TS

Result: 250 days at pH 8 and 6.9 years at pH 7
Test substance: Pentyl 2-hydroxybenzoate (data for structurally related isomer isoamyl salicylate)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (16)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1860000
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08% Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1220
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08%
Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 3800
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08%
Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 0.00050
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08%
Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1550
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08%
Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 608
Aerosol =0.000032% Air =0.86% Fish =0.0053% Sediment =2.08%
Soil =93.6% Suspended Sediment =0.065% Water =3.42%
Test condition: Input parameters: MW, water solubility, calculated log Kow, MP & VP
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (12)

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 0.94; nonlinear - 0.99. Expert survey results: ultimate - 2.9 weeks; primary - 3.8 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate (data for structurally related isomer isoamyl salicylate)
Conclusion: Pentyl 2-hydroxybenzoate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (14)

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Result: other: The test concentration was 29 mg/L. On days 1, 4, 5, 6, 7, 8, 11, 12, 13, 15, 18, 20, 22, 26, and 28, the amount of CO2 formed and degradation was 1.46, 12.27, 9.22, 4.35, 2.18, 2.56, 5.97, 3.47, 3.36, 5.09, 6.77, 6.93, 3.28, 5.67,

Method: other: Commission Directive 79/831/EWG annex V part C; Official Journal of the EC L383 A Part C.4-C "CO2 Evolution Test" (1990) Aerobic
Year: 1993
GLP: yes
Test substance: other TS

Result: Degradation % after time: 29% after 28 days
Time required for 10% degradation: 4 days
Total Degradation: No
Test condition: 10 day window criteria: No
Innoculum: Activated sludge from aeration tank of waste water plant
The tests were performed in duplicate in which 20 mg amyl salicylate/L, a blank control, and 20 mg aniline/L were incubated with the test organism for 28 days at 22+/- 2 C. Evolved CO2 was trapped in BaOH solution that was titrated to determine the amount of CO2 formed.
Test substance: Pentyl 2-hydroxybenzoate (data for structurally related isomer isoamyl salicylate)
Conclusion: Amyl salicylate was classified as not readily biodegradable.
Reliability: (1) valid without restriction
A GLP study conducted under EEC guidelines.
12-AUG-2001 (10)

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Result: other: The BOD and degradation of benzyl salicylate on days 4, 6, 10, 12, 14, 18, 20, 24, 26, and 28 were 98, 118, 141, 151, 156, 166, 168, 172, 174, and 178 mg O2/L, and 46, 55, 66, 71, 73, 78, 79, 81, 82, and 84%, respectively.

Method: other: Council Directive 79/831 EEC Annex V (1990) Method C.4-D "Manometric Respirometry Test" Aerobic
Year: 1995
GLP: no data
Test substance: other TS

Result: Degradation % after time: 84% after 28 days
Time required for 10% degradation: <4 days
Total Degradation: Not within 28 days
Test condition: 10 day window criteria: Yes
Innoculum: Activated sludge from aeration tank of waste water plant
The only modification of the EEC protocol was the use of an

adapted innoculum. The test organism was pretreated through the adaptation of the activated sludge by addition of amyl salicylate for 5 weeks. In a Voith Sapromat, 250 ml of test media containing 100 mg amyl salicylate/L was maintained for 28 days at 20 +/- 1 C using a magnetic stirrer. Any CO2 produced was absorbed in a potassium hydroxide solution. Aniline was used as the reference compound.

Test substance: Pentyl 2-hydroxybenzoate (data for structurally related isomer isoamyl salicylate)

Conclusion: Pentyl 2-hydroxybenzoate (amyl salicylate) was classified as readily biodegradable.

Reliability: (1) valid without restriction
The study conducted in accordance with EEC guidelines.

12-AUG-2001 (11)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Acute Fish Toxicity
Species: other: Zebra fish (Brachydanio rerio)/Hamilton Buchanan/West-Aquarium
Unit: **Analytical monitoring:** yes

Method: other: E.C. Council Directive 92/69/EEC C.1
Year: 1992
GLP: yes
Test substance: other TS

Result: No mortalities at 3.3 mg/L 50% mortality (5/10) at 4.7 mg/L at 24 hours and 100% (10/10) at 48 hours. No mortalities were observed at the five lowest concentrations at 96 hours. Animals at 2.3 and 3.3 mg/L exhibited sluggish swimming action at 48, 72, and 96 hours. Small variations in test conditions were recorded over the 96 hours: Temperature, 22.3-21.3C; [O2] = 7.5-9.1 mg/L; % O2 sat = 88-107%; pH 7.5-8.2.
Undissolved test substance at two highest concentrations

Test condition: Zebra fish (10/group) acclimated for 10 weeks in ISO synthetic water were exposed to 6 concentrations of amyl salicylate (0.8, 1.1, 1.6, 2.3, 3.3, and 4.7 mg/L) under semistatic conditions with daily renewal for 96 hours. The test substance in synthetic water was treated for 60 seconds at 8000 rpm with ultra turrax. Animals were exposed to 12 hours of light in synthetic fresh water. Control and test solutions were monitored for temperature, O2 concentration, % O2 saturation, and pH on a daily basis. The concentration of the test substance was analyzed by GC prior to and after each 24 hours exposure during the first 2 days. Fish were monitored for behavior and mortality daily. Body weight and length were measured at death or after sacrifice at 96 hours.

Test substance: Pentyl 2-hydroxybenzoate

Conclusion: For LC0, mean test concentration was 3.57 mg/L at 0 hours and 0.17 mg/L at 24 or 48 hours. For LC100, mean test concentration was 5.13 mg/L at 0 hours, 24 hours and 0.55 mg/L at 24 and 48 hours. Based on nominal test concentrations, LC0 = 3.9 mg/L, LC100 = 5.5 mg/L, and LC50 = 4.7 mg/L. Based on measured mean concentrations, LC0 = 1.9 mg/L, LC100 = 2.8 mg/L, and LC50 = 2.3 mg/L. Based on mean final concentrations, LC0 = 1.9 mg/L, LC100 = 0.95 mg/L, and LC50 = 1.34 mg/L.

Reliability: (1) valid without restriction
Test protocol comparable to OECD semistatic test protocol.

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Result: 96 hour LC50 = 1.5 mg/L (esters) and 96 hour LC50 = 0.90 mg/L (phenols)
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (15)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: Static 48 hour Daphnia
Species: Daphnia magna (Crustacea)
Unit: **Analytical monitoring:** no data

Method: other: Experimental
Year: 1999
GLP: no data
Test substance: other TS

Test condition: Nominal concentrations as mg/L: 0.5, 1.0, 2.0, 4.0 mg/L
Ten parthenogenetic females per flask
Test substance: Pentyl 2-hydroxybenzoate
16-NOV-2001 (6)

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Conclusion: 48 hour LC50 = 0.9 (esters) and 48 hour LC50 = 1.1 mg/L (phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (15)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Pentyl 2-hydroxybenzoate
Conclusion: 96 hour EC50 = 0.13 (esters) and 96 hour EC50 = 0.65 mg/L (phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (15)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data

Method: other: LD50 calculated
Year: 1982
GLP: no
Test substance: other TS

Remark: The principal toxic signs reported included lethargy, chromorhinorrhea, ptosis, and chromodacryorrhea. At necropsy, the survivors were considered generally normal. Animals dying during the study were reported to have lung, heart, liver, stomach, kidney and intestinal abnormalities.
Result: LD50 = 4100 (3300-5000) mg/kg bw
Number of deaths at each dose level: 4/10, 4/10, 7/10, and 10/10 deaths at 3100, 4000, 5000, and 6300 mg/kg bw, respectively.
Test condition: Groups of 10 rats were orally administered (method not stated) 3.1, 4.0, 5.0, or 6.3 g/kg bw of test substance.
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results.Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (8)

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 2
Vehicle: other:50% alcohol in polyethylene glycol 200 v/v
Route of admin.: other: Gavage

Method: LD50 calculated by using Hazleton protocol number P7/152 and OECD Guidelines for Testing Chemicals May 12, 1981
Year: 1982
GLP: no
Test substance: other TS

Remark: The only signs reported were "shaggy coat" following treatment and "emaciation" on days 3 and 4. All animals appeared normal by day 5 and there was reported body weight

	gain by the end of the observation period. No macroscopic lesions were found.
Result:	LD50 = >5,000 mg/kg bw Number of deaths at each dose level: There were no deaths at any of the doses in either the dose range-finding or main studies.
Test condition:	In the dose range-finding study, groups of 2 male and 2 female rats were gavaged with 500, 800, 1,260, 2,000 or 5,000 mg isoamyl salicylate/kg bw following an overnight fast and observed for 7 days. At the end of 7 days all surviving animals were killed. In the main study, 5 male and 5 female rats were gavaged with 5,000 mg isoamyl salicylate/kg bw and then observed for 14 days. Body weights were measured on day 0, 7 and 14. At the end of 14 days all surviving animals were killed by CO2 inhalation and the animals were examined macroscopically.
Test substance:	Pentyl 2-hydroxybenzoate
Reliability:	(1) valid without restriction This study was conducted at a reputable laboratory following OECD guidelines and therefore the data are considered reliable.

30-JUN-2001 (7)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data

Method: other: LD50 calculated
Year: 1982
GLP: no
Test substance: other TS

Remark: The principal toxic signs reported included alopecia, few feces, lethargy, and yellow nasal discharge. Skin reactions were graded as severe/slight. At necropsy, the animals were reported to have lung and intestinal abnormalities as well as alopecia.
Result: LD50 = >5000 mg/kg bw
Number of deaths at each dose level: 0/10 deaths at 5000 mg/kg bw
Test condition: Ten rabbits were topically administered 5 g/kg bw of test substance.
Test substance: Pentyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results.Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (9)

5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: Wistar
Route of administration: oral feed
Exposure period: 13 weeks; additional groups 2 or 6 weeks
Frequency of treatment: ad libitum
Post exposure period: None
Doses: 0, 50, 500 or 5000 ppm; additional groups received: 0, 500, or 5000 ppm Actual dose: approximately 0, 4.7, 46, or 415 mg/kg bw/d for males and 0, 4.8, 46.9, or 475 mg/kg bw/d for females
Control Group: other: basal diet only

Method: other: 13-week feeding study
Year: 1975
GLP: no
Test substance: other TS

Remark: Statistical evaluations: Yes. Student's t-test, the ranking method of White (1952) and Lord's range test (Langley, 1968) The reduction in body weight gain appeared to be due to the poor palatability of the test diet as indicated by the reduced food consumption and supported by the results of the paired-feeding study. The authors suggested that the increased volumes of urine with a lower specific gravity in high-dose females may have been a result of a functional injury to the kidney [not reported in males]; however, this was not supported by histopathological examination of the kidneys. The slight increase in relative kidney weights was not seen at 6 weeks and was not dose-related in the males (only increased at the mid-dose in males and at the high-dose in females) at 13 weeks. Although the authors indicated that this may be a toxic effect on the kidney, with the absence of histopathological changes, no dose-related effect in males and only a minor, although significant, increase in high-dose females, this conclusion is difficult to ascertain. The increased relative liver weight noted in high-dose females was not accompanied with histopathological changes and the authors

Result: LOAEL: 5000 ppm
NOAEL: 500 ppm
Toxic response/effects by dose level: At the high-dose, both sexes showed statistically significant decreases in body weight gain from day 1. Food consumption also was significantly decreased in high-dose animals. Water consumption was significantly decreased on day 1 in all animals fed isoamyl salicylate, but was comparable to controls in treated males and was significantly increased in high-dose females. Approximately half of the high-dose animals showed signs of respiratory infection from week 3 which regressed in all cases but one female. The female was killed during week 6 and necropsied showing mucus and pus cells in the bronchioles and renal tubular dilatation. Hematology results showed no consistent findings. Urinalysis showed a lower specific gravity of urine from high-dose rats which was only significant ($P<0.05$) at week 6 for both sexes and at week 13 for females. A 6-hour collection taken during week 2 showed females producing an increased urine volume. Organ weights showed sporadic differences from controls which were not consistently obs

Test condition: Groups of 15 male and 15 female Wistar rats were fed diets containing 0, 50, 500, or 5000 ppm isoamyl salicylate (approximately 0, 4.7, 46, or 415 mg/kg bw/d for males and 0, 4.8, 46.9, or 475 mg/kg bw/d for females, respectively) for a period of 13 weeks. Animals were regularly observed for clinical signs, behaviour, and condition. Body weight, and food and water consumption were also measured. During the last 2 days of exposure, urine samples were collected

for urinalysis. At the end of the study, blood samples were taken for hematological examination and necropsies were performed and the following organs were weighed and samples of which were preserved: brain, heart, liver, stomach, small intestine, cecum, spleen, kidneys, adrenal glands, gonads, pituitary and thyroid. In addition, samples of lung, lymph nodes, salivary gland, trachea, esophagus, aorta, thymus, urinary bladder, colon, rectum, pancreas, uterus and skeletal muscle were taken and preserved. Histopathological examination was conducted only on high-dose animals, half of the controls and h

Test substance: Pentyl 2-hydroxybenzoate (data for structurally related substance, isoamyl salicylate)

Conclusion: Isoamyl salicylate produced no adverse effects in rats fed up to 500 ppm in the diet over 13 weeks.

Reliability: (2) valid with restrictions
The study was conducted prior to GLP but followed standard methodologies and was published in a peer reviewed journal.

07-AUG-2001 (4)

5.5 Genetic Toxicity 'in Vitro'

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5.6 Genetic Toxicity 'in Vivo'

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5.8.2 Developmental Toxicity/Teratogenicity

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5.8.3 Toxicity to Reproduction, Other Studies

-

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IUCLID Data Set

Existing Chemical ID: 118-58-1
CAS No. 118-58-1
EINECS Name benzyl salicylate
EC No. 204-262-9
Molecular Formula C14H12O3

Producer Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Substance Related Part
Company: Aromatic Consortium, The Flavor and Fragrance High Production Volume Consortia
Creation date: 28-JUN-2001

Memo: Benzyl Derivatives

Printing date: 16-NOV-2001
Revision date:
Date of last Update: **16-NOV-2001**

Chapter (profile): Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.2, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8.2, 5.8.3
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 Melting Point

Value: = 130.5 degree C

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (16)

2.2 Boiling Point

Value: = 208 degree C at 33 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (8)

Value: = 320 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (1)

Value: = 335 degree C at 1013 hPa

Method: other: Measured
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are from a recognized literature source and are considered reliable.
16-NOV-2001 (3)

2.4 Vapour Pressure

Value: = .0013 hPa at 20 degree C

Method: other (measured)
GLP: no data
Test substance: other TS

Method: Measured
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (1) valid without restriction
The data are obtained by a recognized literature source and are consistent with chemical structure.
16-NOV-2001 (4)

Value: = .000024 hPa at 25 degree C

Method: other (calculated)
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a measured boiling point of 320 C.
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (15)

2.5 Partition Coefficient

log Pow: = 4.31 at 25 degree C

Method: other (measured)
GLP: no data

Method: Calculated
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (14)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 24.59 mg/l at 25 degree C

Method: other
GLP: no data
Test substance: other TS

Method: Calculated
Test condition: Calculated based on a log Kow = 4.31
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.

16-NOV-2001 (17)

3.1.1 Photodegradation

DIRECT PHOTOLYSIS
Halflife t1/2: = 7.4 hour(s)

GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (10)

3.1.2 Stability in Water

Type: abiotic

Method: other: Calculated Aqueous Base/Acid catalyzed hydrolysis
GLP: no data
Test substance: other TS

Result: 63 days at pH 8 and 1.7 years at pH 7
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation method and are consistent with chemical structure.
16-NOV-2001 (13)

3.3.2 Distribution

Media: other: Aerosol-Air Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2.26E+08
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06% Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

Media: other: Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 804
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06%
Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

Media: other: Suspended Sediment-Water Partition Coefficient
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 2510
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06%
Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

Media: water - air
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1.46E-05
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06%
Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

Media: water - biota
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 1020
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06%
Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

Media: water - soil
Method: Calculation according Mackay, Level I

Result: Absorption coefficient: 402
Aerosol =0.00017% Air =0.037% Fish =0.0052% Sediment =2.06%
Soil =92.7% Suspended Sediment =0.064% Water =5.13%
Test condition: Input parameters: MW, MP & calculated VP, log Kow, water solubility
Model conditions: 25 C, 100,000 lbs.
Reliability: (4) not assignable
The data are obtained by a recognized fugacity calculation method. Data are considered
16-NOV-2001 (9)

3.5 Biodegradation

Type: aerobic
Result: other: Probability of rapid biodegradation: linear model - 1.06; nonlinear - 0.999. Expert survey results: ultimate - 2.9 weeks; primary - 3.8 days.

Method: other: Calculated MITI model
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Conclusion: Benzyl 2-hydroxybenzoate is predicted to be readily degradable.
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (11)

Type: aerobic
Inoculum: activated sludge
Contact time: 28 day(s)
Result: other: The BOD and degradation of benzyl salicylate on days 2, 6, 8, 14, 16, 20, 22, 26, and 28 were 81, 161, 171, 185, 187, 193, 195, 196, and 196 mg O2/L, and 36, 70, 75, 81, 82, 85, 86, 87, and 87%, respectively.

Method: other: Council Directive 79/831 EEC Annex V (1990) Method C.4-D "Manometric Respirometry Test" (essentially identical to OECD Guideline 301F Aerobic
Year: 1992
GLP: yes
Test substance: other TS

Result: Degradation % after time: 87% after 28 days
Time required for 10% degradation: <2 days
Total Degradation: Not within 28 days
Test condition: 10 day window criteria: Yes
In a Voith Sapromat, 250 ml of test media containing 100 mg benzyl salicylate/L was maintained for 28 days at 20 +/- 1 C using a magnetic stirrer. Any CO2 produced was absorbed in a potassium hydroxide solution. Aniline was used as the reference compound.
Innoculum: Activated sludge from aeration tank of waste water plant
Test substance: Benzyl 2-hydroxybenzoate
Conclusion: Benzyl salicylate was classified as readily biodegradable.
Reliability: (1) valid without restriction
The GLP study conducted in accordance with EEC and OECD guidelines.
12-AUG-2001 (7)

Type: aerobic
Inoculum: other: Secondary effluent from treatment unit collected the night on the day of the test.
Contact time: 28 day(s)
Result: other: Benzyl salicylate underwent 62% biodegradation after 28 days.

Method: other: ISO Method 14593 CO2 evolution test
Year: 1999
GLP: yes
Test substance: other TS

Result: Degradation % after time: 62% after 28 days
Total Degradation: No
Test condition: 10 day window criteria: No
Nominal test concentration was 10 mg DOC/L at 20C for 28 days. The reference substance was aniline. Biodegradation was monitored on days 4, 7, 14, 21, and 28. pH of test medium was 7.0=/-0.2.
Test substance: Benzyl 2-hydroxybenzoate
Conclusion: Benzyl salicylate is classified as readily biodegradable.

Reliability: (2) valid with restrictions
The data are reported in the form of an abstract, however, the study was conducted according to a recognized protocol and the results are consistent with chemical structure.
27-APR-2001 (6)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: Acute Fish Toxicity
Species: other: Zebra fish (Brachydanio rerio)/Hamilton Buchanan/West-Aquarium
Unit: **Analytical monitoring:** yes

Method: other: E.C. Council Directive 92/69/EEC C.1
Year: 1993
GLP: yes
Test substance: other TS

Result: No mortalities at 0.7, 1.0, 1.4 mg/L after 96 hours. 100% mortality (10/10) at 2.0 and 2.4 mg/L at 24 hours. Animals at 2.0 and 2.4 mg/L exhibited normal swimming action during test. Small variations in test conditions were recorded over the 96 hours: Temp, 22.-19.5C; [O2] = 8.4-10.5 mg/L; % O2 sat = 93-119%; pH = 7.6-8.1.

Test condition: Zebra fish (10/group) acclimated for 10 weeks in ISO synthetic water were exposed to 6 concentrations of benzyl salicylate (0.7, 1.0, 1.4, 2.0, and 2.8 mg/L) under semistatic conditions with daily renewal for 96 hours. The test substance in synthetic water was treated for 60 seconds at 8000 rpm with ultra turrax. Animals were exposed to 12 hours of light in synthetic fresh water. Control and test solutions were monitored for temperature, O2 concentration, % O2 saturation, and pH on a daily basis. The concentration of the test substance was analyzed by GC prior to and after each 24 hours exposure during the first 2 days. Fish were monitored for behavior and mortality daily. Body weight and length were measured at death or after sacrifice at 96 hours.

Test substance: Benzyl 2-hydroxybenzoate

Conclusion: For LC0, mean test concentration was 1.23 mg/L prior to and 0.35 mg/L 24 hours after treatment. For LC100, test concentration was 1.82 mg/L prior to and 0.87 mg/L after treatment on first day. Based on nominal test concentrations, LC0 = 1.4 mg/L, LC100 = 2.0 mg/L, and LC50 = 1.7 mg/L. Based on measured mean concentrations, LC0 = 0.84 mg/L, LC100 = 1.7 mg/L, and LC50 = 1.2 mg/L. Based on mean final concentrations, LC0 = 0.35 mg/L, LC100 = 0.87 mg/L, and LC50 = 0.55 mg/L.

Reliability: (1) valid without restriction
Test protocol comparable to OECD semistatic test protocol.

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Result: 96 hour LC50 = 2.0 mg/L (esters) & 1.3 mg/L (phenols)
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
17-MAY-2001 (12)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: other:
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Conclusion: 48 hour LC50 = 1.4 (esters) and 48 hour LC50 = 1.4 mg/L phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (12)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Unit: **Analytical monitoring:** no data

Method: other: Calculated
GLP: no data
Test substance: other TS

Test substance: Benzyl 2-hydroxybenzoate
Conclusion: 96 hour EC50 = 0.17 (esters) and 96 hour EC50 = 1.02 mg/L (phenols)
Reliability: (4) not assignable
The data are obtained by a recognized SAR calculation and are consistent with chemical structure.
16-NOV-2001 (12)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

Type: LD50
Species: rat
Strain: no data
Sex: male
No. of Animals: 6
Vehicle: no data
Route of admin.: other: Gavage

Method: LD50 calculated by using the Thompson Moving average method
Year: 1970
GLP: no
Test substance: other TS

Remark: The principal toxic effect was reported to be depression.
Result: LD50 = 2,227 mg/kg bw (1,925 - 2,580)
Number of deaths at each dose level: There were 0, 4, and 6 deaths in the 1,250, 2,500, and 5,000 mg/kg bw dose groups, respectively.
Test condition: Groups of 6 male rats received a single dose of 1,250, 2,500 or 5,000 mg benzyl salicylate/kg bw after fasting for 18 hours. Animals were observed for 7 days.
Test substance: Benzyl 2-hydroxybenzoate
Reliability: (2) valid with restrictions
Data reported in a brief memo with limited description of protocol and results. Data were collected prior to GLP and OECD guidelines but by a method comparable to present guidelines/standards and under direction of a recognized research institute. Even though details are not available, the data are considered reliable.
30-JUN-2001 (2)

5.1.2 Acute Inhalation Toxicity

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5.1.3 Acute Dermal Toxicity

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5.1.4 Acute Toxicity, other Routes

5.4 Repeated Dose Toxicity

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5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: bacterial Salmonella typhimurium TA100, TA1535, TA1537 and TA98
Concentration: Lab 1: 0, 0.3, 1.0, 3.3, 10, 20, 33, 100, 333, or 666 ug/plate; Lab 2:0, 3.3, 10, 33.3, 100, or 333.3 ug/plate
Metabolic activation: with
Result: positive

Method: other: Ames assay (modified) (Haworth et al., 1983)
Year: 1987
GLP: no
Test substance: other TS

Result: Benzyl salicylate showed no mutagenic activity in any of the strains tested with or without S9.
Test condition: Metabolic activation: 10% S9 fractions of Aroclor 1254-induced male SD rat and male Syrian hamster livers Toxicity was tested in a preliminary test with TA100. DMSO was used as the solvent. Benzyl salicylate was incubated with Salmonella without shaking at 37 C for 20 min. The test solution was plated onto petri dishes containing Vogel-Bonner medium and revertant colonies were machine-counted following a 2-day incubation period. Positive controls used were sodium azide, 9-aminoacridine, 4-nitro-o-phenylenediamine, and 2-aminoanthracene. Benzyl salicylate was tested in 2 separate laboratories as a coded substance [blind test]. A result was considered positive "if it produced a reproducible, dose-related increase in his+ revertants over the corresponding solvent controls in replicate trials." Only after the results were determined was the identity of the tested chemical revealed.
Test substance: Benzyl 2-hydroxybenzoate
Conclusion: Benzyl 2-hydroxybenzoate was non-mutagenic in this assay.
Reliability: (1) valid without restriction
Study was conducted using standard methodology and published in a peer-reviewed journal.
07-AUG-2001 (18)

5.6 Genetic Toxicity 'in Vivo'

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5.8.2 Developmental Toxicity/Teratogenicity

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5.8.3 Toxicity to Reproduction, Other Studies

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